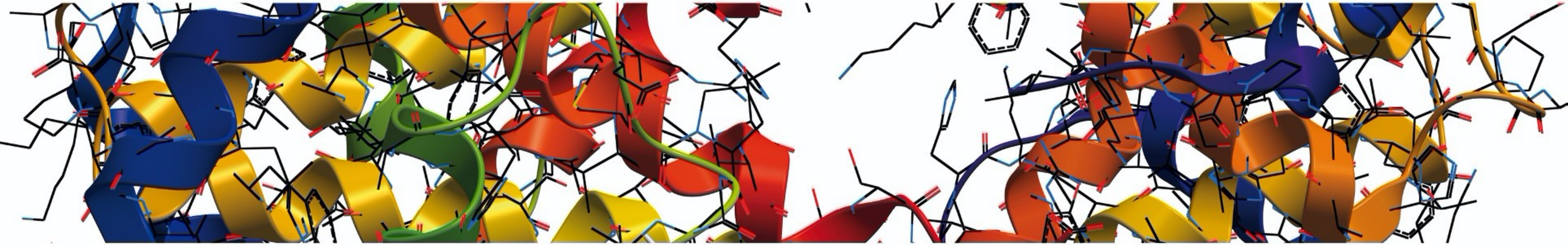
AGGIORNAMENTO SU DIAGNOSI E TERAPIA DELLE EMOGLOBINOPATIE

Ferrara, 4 luglio 2025 | Hotel Ferrara

Diagnostica delle emoglobinopatie

Giorgia Mandrile



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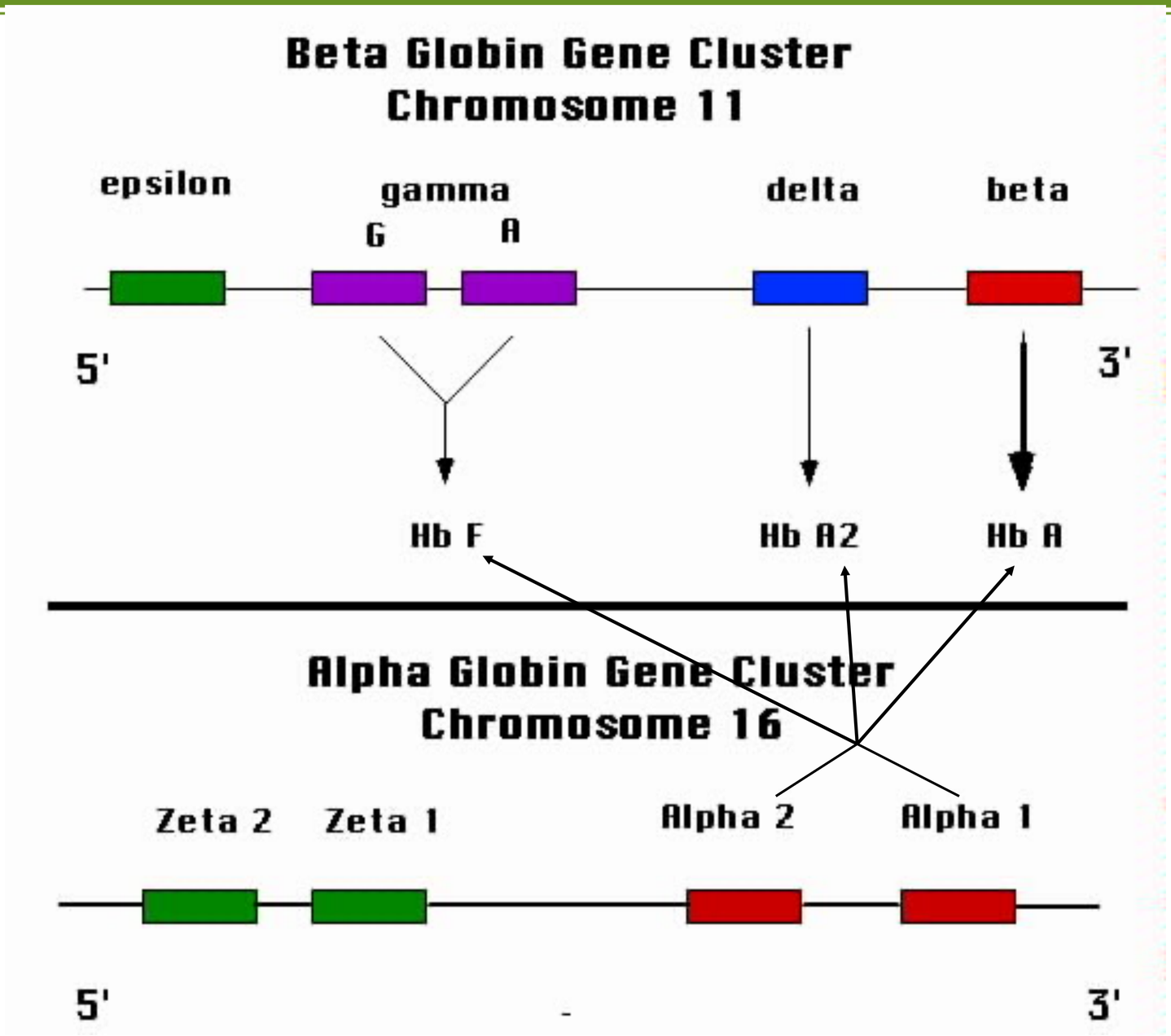
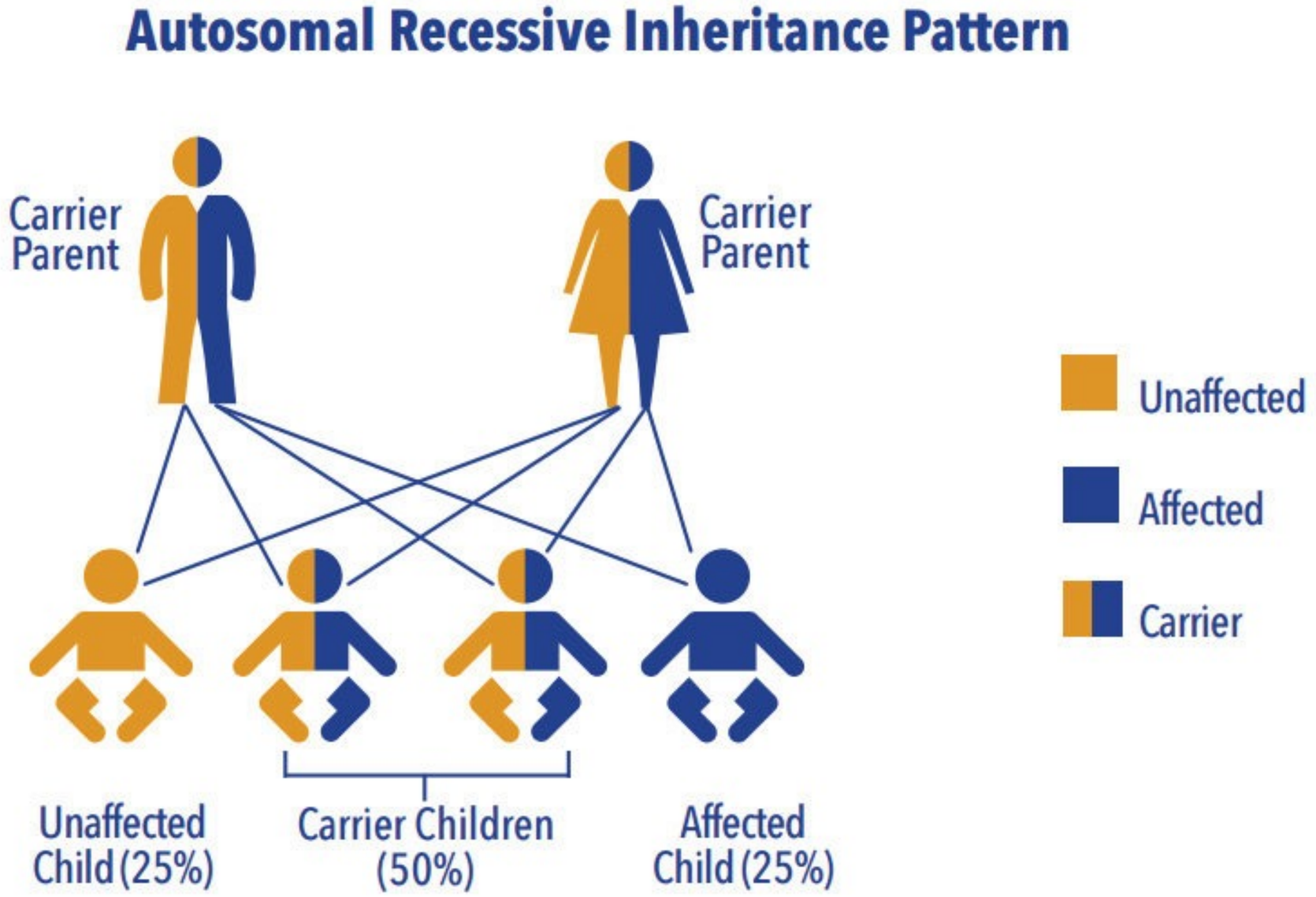
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Alnylam	x		x				
Novo Nordisk	x		x				


Beta Globin Gene Cluster
Chromosome 11
epsilon
gamma
G
A
delta
beta
5'
3'
Hb F
Hb A2
Hb A
Alpha Globin Gene Cluster
Chromosome 16
Zeta 2
Zeta 1
Alpha 2
Alpha 1
5'
3'


Autosomal Recessive Inheritance Pattern
Carrier Parent
Carrier Parent
Unaffected
Affected
Carrier
Unaffected Child (25%)
Carrier Children (50%)
Affected Child (25%)

Ematochimici

Diagnostica di I e II livello delle Emoglobinopatie

Buone Pratiche SITE

Genetici

European Journal of Human Genetics (2015) 23, 426–437

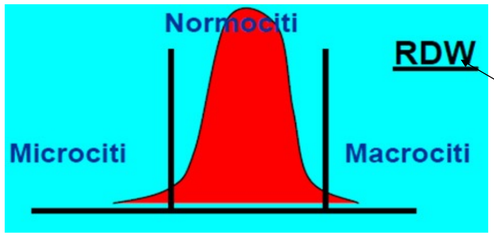
www.nature.com/ejhg

EJHG Open

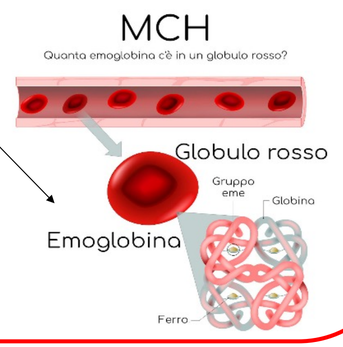
POLICY

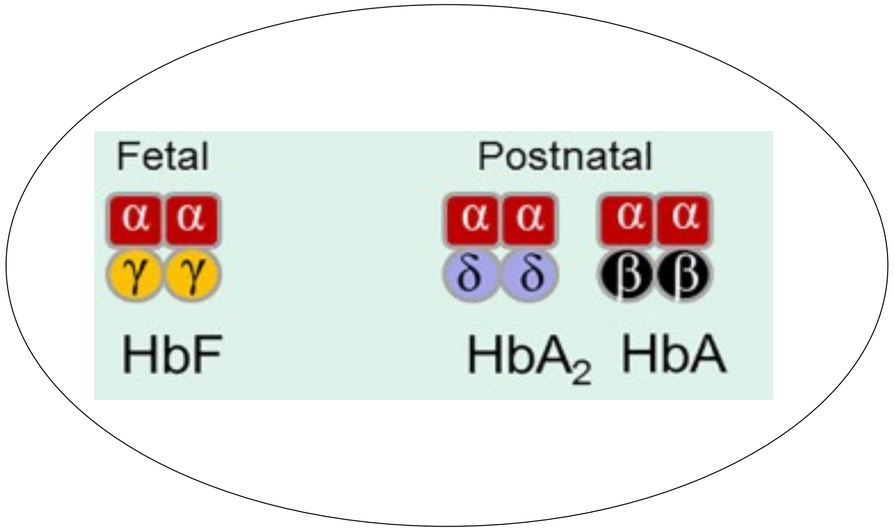
EMQN Best Practice Guidelines for molecular and haematology methods for carrier identification and prenatal diagnosis of the haemoglobinopathies

SITE SOCIETA' ITALIANA TALASSEMIE ED EMOGLOBINOPATIE


Normociti
RDW
Microciti
Macrociti

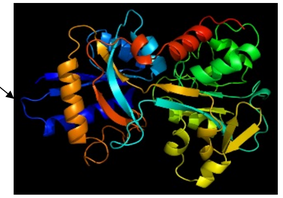

MCV tra
80-100 fl

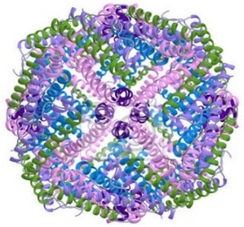

MCH
Quanta emoglobina c'è in un globulo rosso?
Globulo rosso
Gruppo eme
Globina
Emoglobina
Ferro


Fetal
Postnatal
HbF
HbA2
HbA


Serum Iron Test


26
Fe
Ferro
55.845





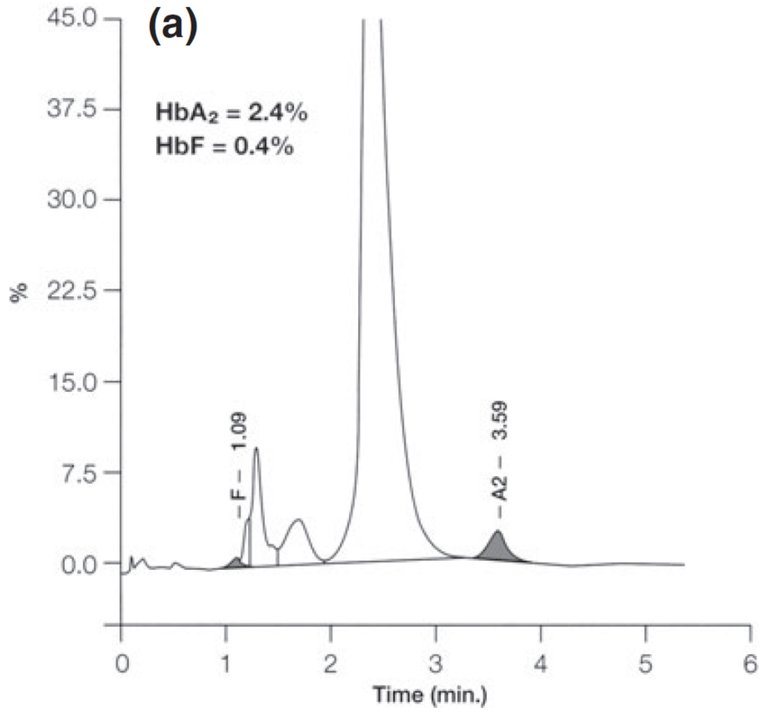
Hb	13,9
MCV	71,6
MCH	24,1
RDW	17,6
Serum Iron	36
Transferrin	335
Transferrin sat	7%
Ferritin	4
HbA2	2,6
HbF	0,1

Hb	16,6
MCV	88
MCH	27,6
RDW	16,3
Serum Iron	123
Transferrin	276
Transferrin sat	33%
Ferritin	56
HbA2	2,6
HbF	0,1

Globin separation

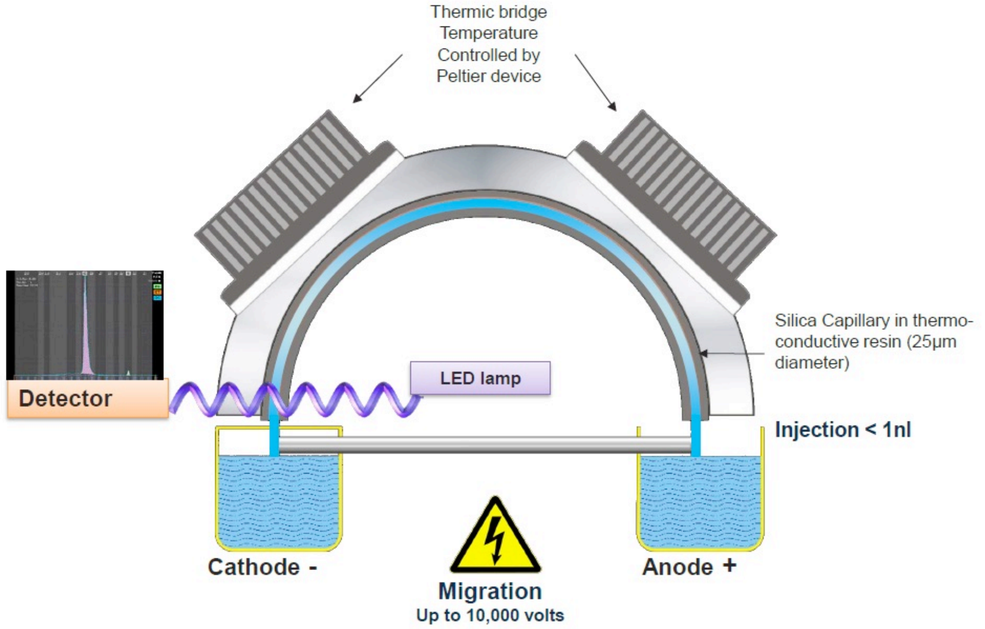
High Performance Liquid Chromatography (HPLC)

Column with small silica spheres, weakly cationic.

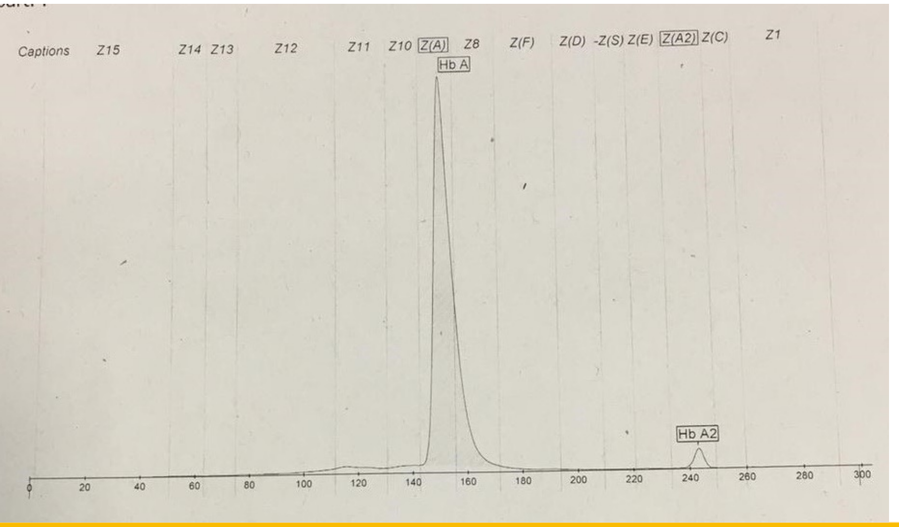


Capillary electrophoresis (CE)

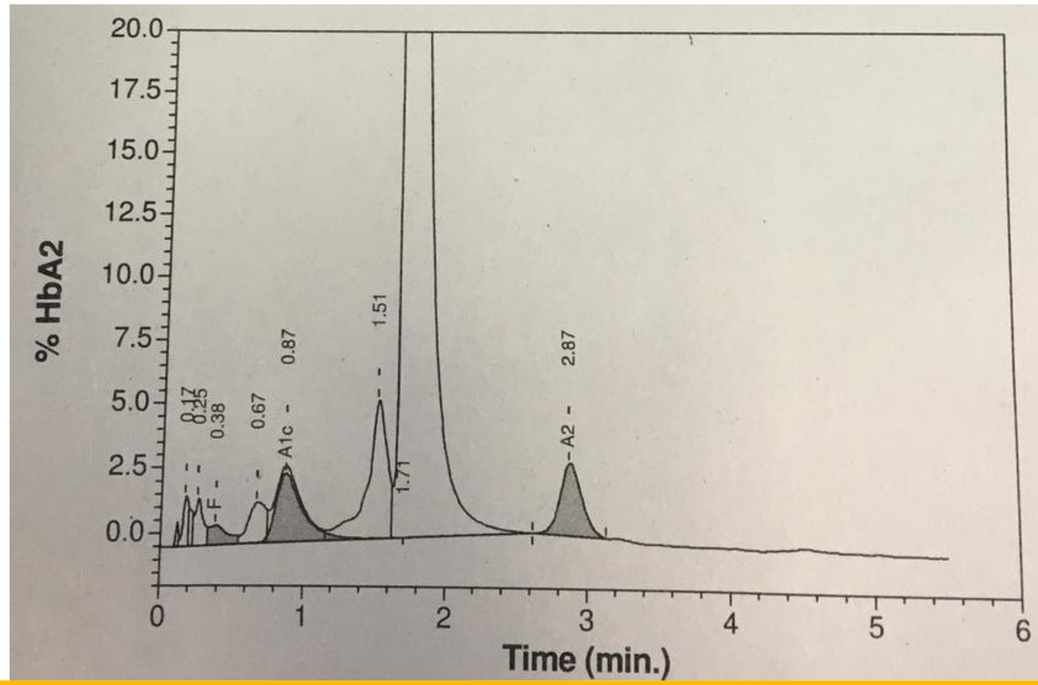
Charged molecules are separated by their electrophoretic mobility

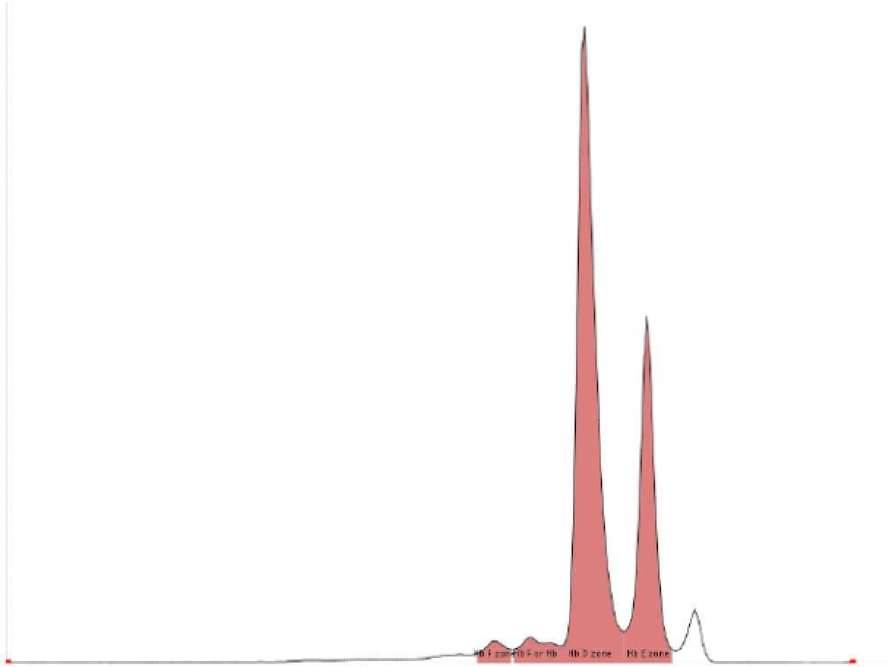


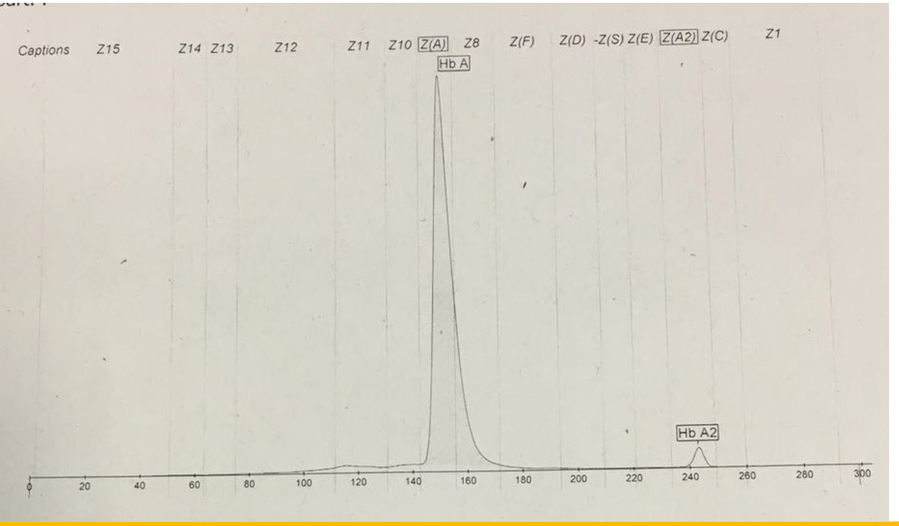
CE

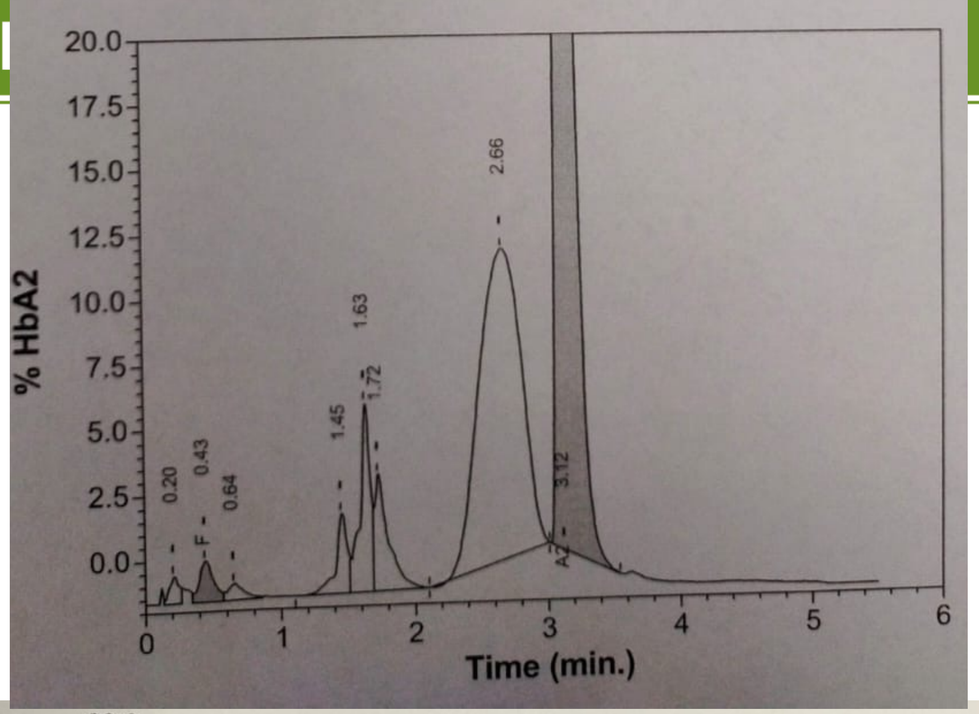

Captions
Z15
Z14 Z13
Z12
Z11
Z10 Z(A)
Z8
Z(F)
Z(D) -Z(S) Z(E) Z(A2) Z(C)
Z1
Hb A
Hb A2
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300

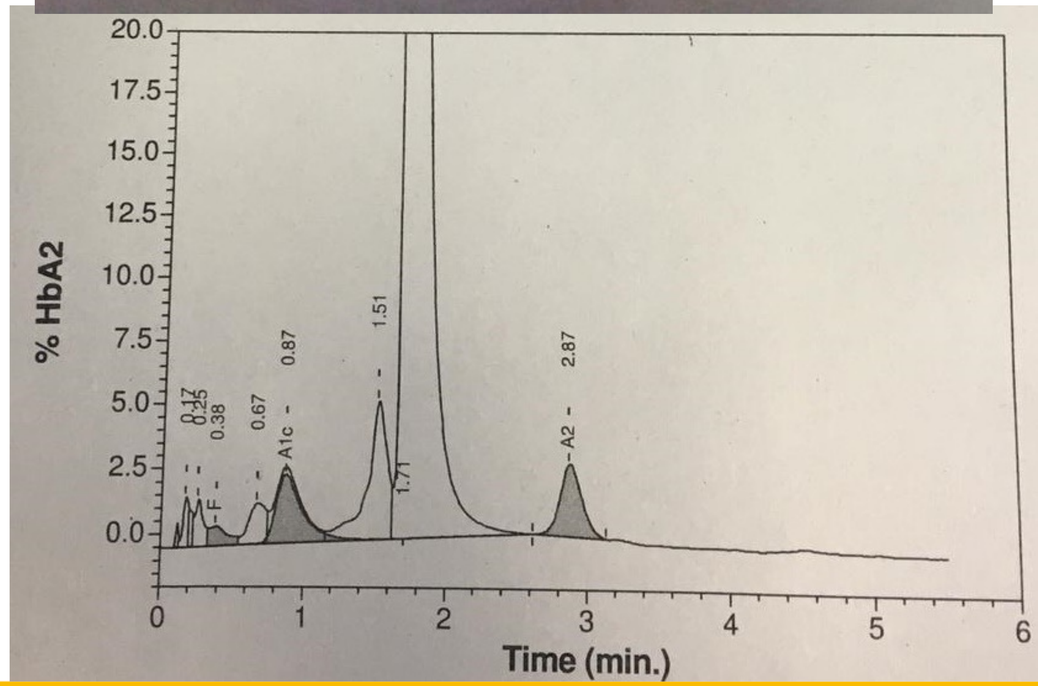
HPLC

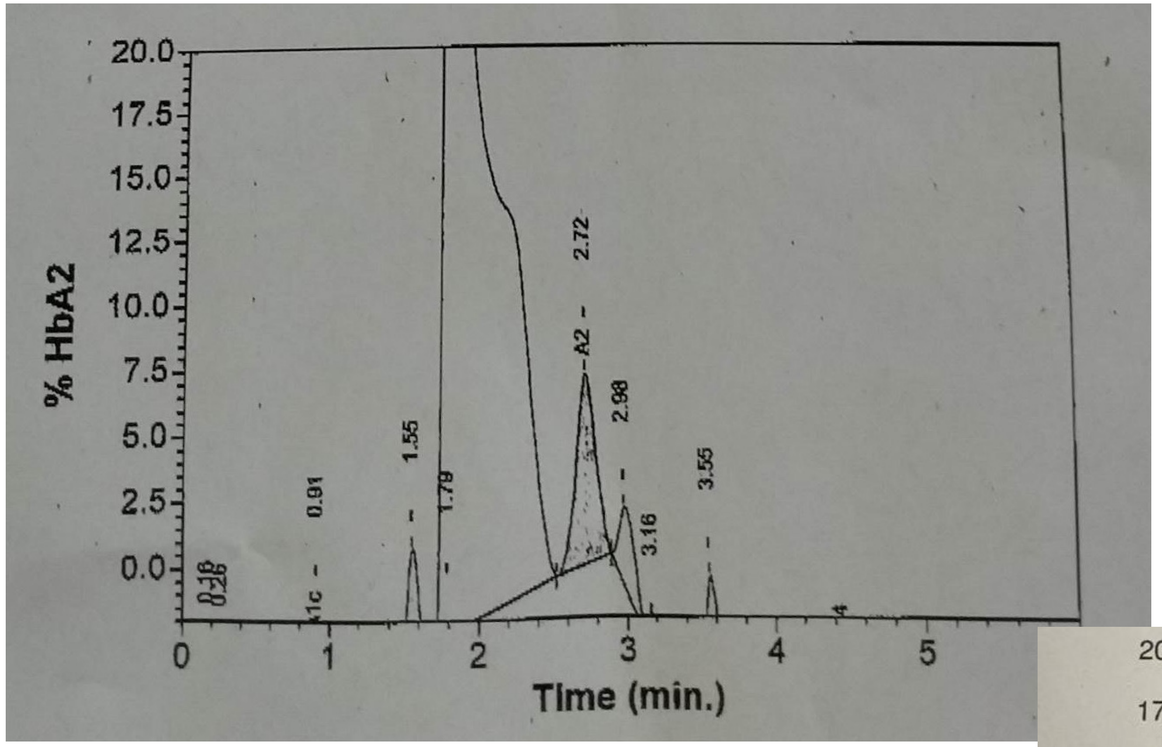

% HbA2
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0.17
0.25
0.38
F
0.67
A1c
0.87
1.51
1.71
A2
2.87
0
1
2
3
4
5
6
Time (min.)

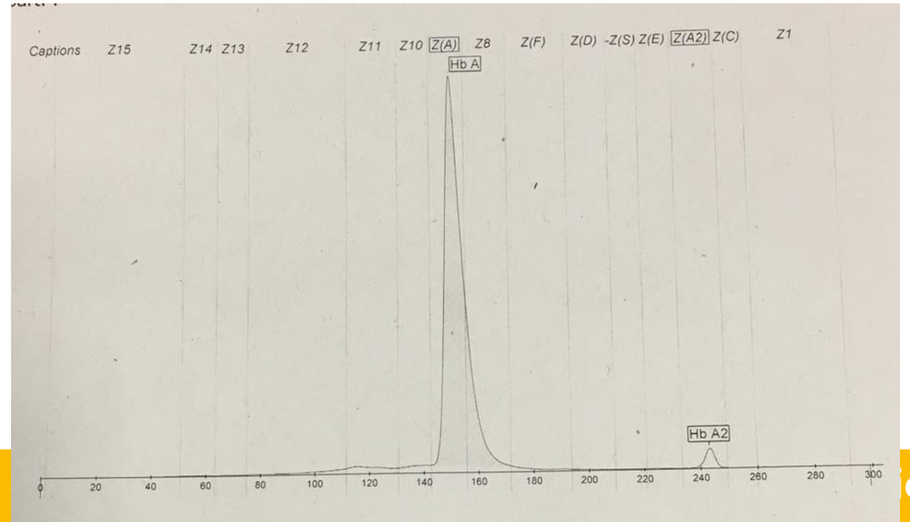


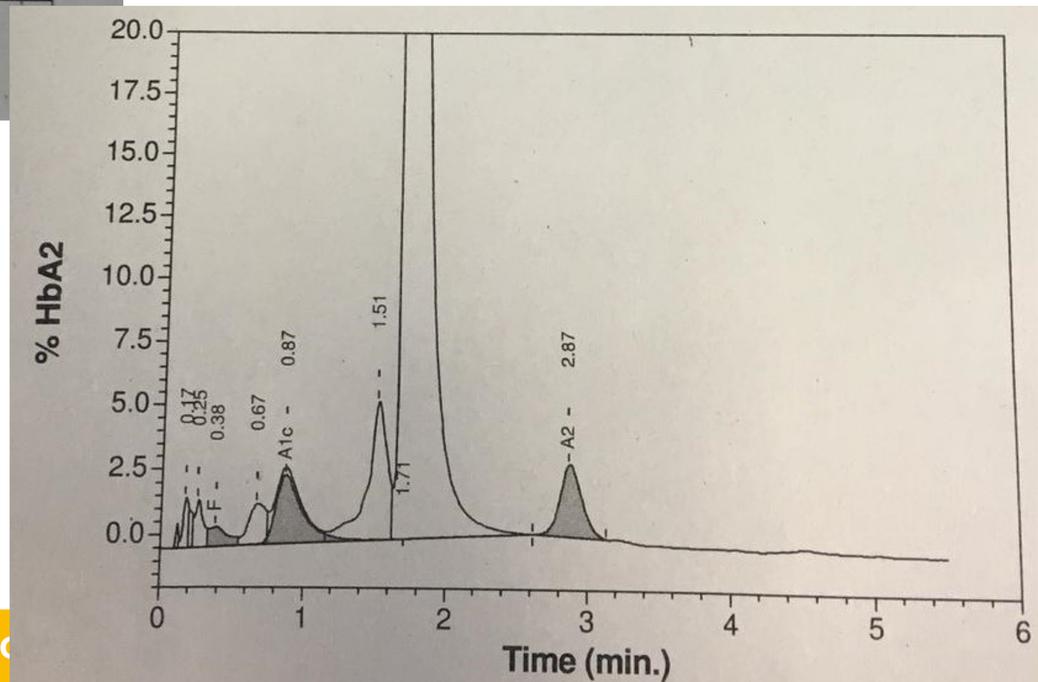

Captions Z15 Z14 Z13 Z12 Z11 Z10 Z(A) Z8 Z(F) Z(D) -Z(S) Z(E) Z(A2) Z(C) Z1
Hb A
Hb A2
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300


% HbA2
20.0 17.5 15.0 12.5 10.0 7.5 5.0 2.5 0.0
0.20 0.43 F 0.64 1.45 1.63 1.72 2.66 A2 3.12
0 1 2 3 4 5 6
Time (min.)


% HbA2
20.0 17.5 15.0 12.5 10.0 7.5 5.0 2.5 0.0
0.17 0.25 0.38 F 0.67 A1c 0.87 1.51 1.71 A2 2.87
0 1 2 3 4 5 6
Time (min.)


% HbA2
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0.16
0.26
A1c
0.91
1.55
1.79
A2
2.72
2.98
3.16
3.55
0
1
2
3
4
5
Time (min.)


Captions
Z15
Z14
Z13
Z12
Z11
Z10
Z(A)
Z8
Z(F)
Z(D)
Z(S)
Z(E)
Z(A2)
Z(C)
Z1
Hb A
Hb A2
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300


% HbA2
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0.17
0.25
F
0.38
0.67
A1c
0.87
1.51
1.71
A2
2.87
0
1
2
3
4
5
6
Time (min.)

Normal

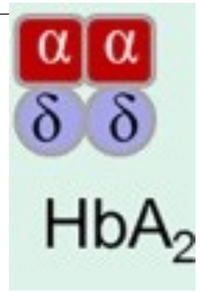
12 – 15 g/dl

13 – 17 g/dl

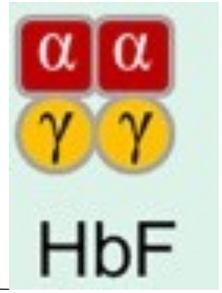
MCV 80 – 100 fl

MCH 27 – 31 pg

In adults
AGE DEPENDENT!



2 – 3,2 %

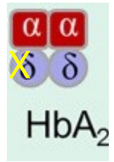


<1,5 %

Delta thalassemia carrier

12 – 15 g/dl

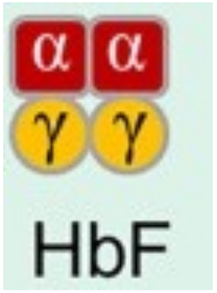
13 – 17 g/dl



1 – 1,5 %

MCV 80 – 100 fl

MCH 27 – 31 pg



0 – 1,5 %

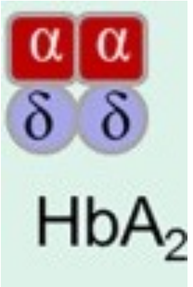
Hereditary Persistance Fetal Hb (HPFH)

12 – 15 g/dl

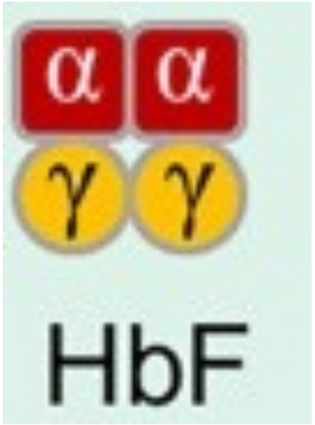
13 – 17 g/dl

MCV 80 – 100 fl

MCH 27 – 31 pg



2 – 3,2 %



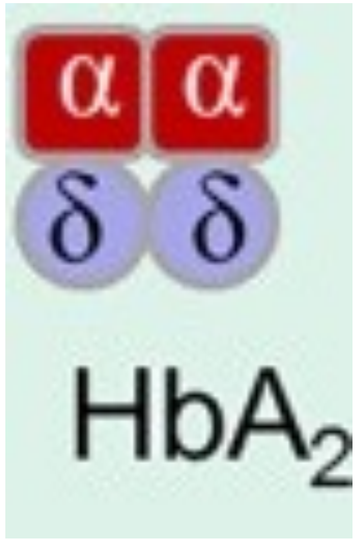
>1,5 %

Beta thalassemia carrier

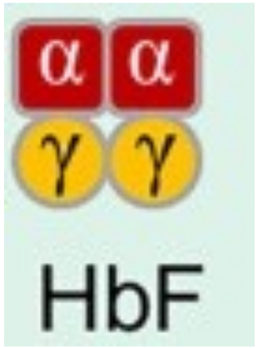
Mutation dependent

MCV 60 - 75 fl

MCH 18 – 26 pg



>3,5%



>1,5 %

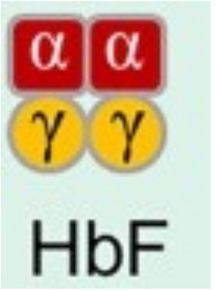
Beta +

Mutation dependent

MCV 80 – 100 fl

MCH 27 – 31 pg

3,2 – 3,5 %



0 – 1,5 %

Known causes underlying Hb A_2 levels outside the normal range

Increase (excluding β-thalassaemia variants)

Genetic

KLF1 variants

Triplicated α gene

Some unstable variants

Hb variants eluting with or close Hb A_2

Acquired

Hyperthyroidism

Megaloblastic anaemia

Aplastic crisis in HS

Antiretroviral drugs

Pseudoanthoma elasticum

Reduction

Genetic

δ-thalassaemia

δ-chain variants

α-chain variants

Hb Lepore[1]

α-thalassaemia[2]

δβ and γδβ thalassaemia;

some mild β thalassaemia variants

Acquired

Severe iron deficiency anaemia

Sideroblastic anaemia

Lead poisoning

Leukaemia, aplastic anaemia

EMQN Best Practice Guidelines for molecular and haematology methods for carrier identification and prenatal diagnosis of the haemoglobinopathies

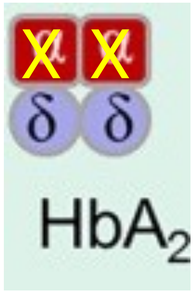
Alpha thalassemia carrier

12 – 15 g/dl

13 – 17 g/dl

MCV ≤ 80 fl

MCH <27 pg



2 – 3,2 %

0 – 1,5 %

Alpha thalassemia carrier

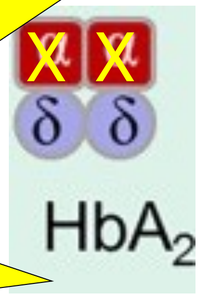
12 – 15 g/dl

13 – 17 g/dl

MCV ≤ 80 fl

MCH <27 p

4 alpha genes!!



2 – 3,2 %

0 – 1,5 %

Standardizzazione dei valori di laboratorio per la diagnosi di portatore di alfa talassemia

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[2] Epidemiologia dei tumori e CRPT U, Medicina d'Urgenza – MECAU, Dipartimento Scienze Mediche, Università di Torino, AOU Città della Salute e della Scienza di Torino – Presidio Molinette

Introduzione: La maggior parte delle linee guida ritengono un MCV < 79 fl e un MCH < 27 pg utili allo screening diagnostico di portatore sano di alfa talassemia e suggeriscono l'identificazione di valori propri di riferimento.

Obiettivo dello Studio: verificare la sensibilità/specificità di tali indicazioni nella nostra realtà ed eventualmente individuare nuovi valori soglia di MCV e MCH ottimali per l'orientamento diagnostico di portatore sano di alfa talassemia nella nostra attività di prevenzione.

Metodi: abbiamo incluso nell'analisi solo i soggetti studiati direttamente a livello molecolare in quanto partner di portatori sani di beta talassemia e HbS afferenti all'ambulatorio di diagnosi e prevenzione delle emoglobinopatie, nel periodo 2011-2018.

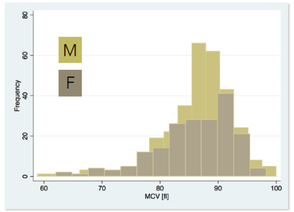
La diagnosi molecolare è stata effettuata con reverse dot blot o sequenziamente del gene.

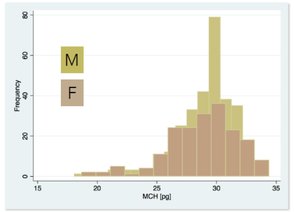
Criteri di esclusione: soggetti selezionati per lo studio molecolare in base agli indici globulari o a criteri clinici; soggetti portatori (o con associazione di) di altra emoglobinopatia o di triplo alfa; soggetti con valori di saturazione della transferrina < 15%, o ferritina < 30 ng/dl; soggetti con patologie croniche.

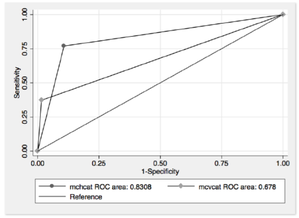
È stata calcolata l'accuratezza diagnostica per MCV < 80 fl e MCH < 27 pg.

Per individuare i valori di MCV e MCH per massimizzare sensibilità e specificità dei parametri abbiamo applicato il test di Youden e il metodo di Liu).

Risultati: da un totale di 489 pazienti analizzati, sono state incluse 394 osservazioni: l'età mediana era di 33,5 anni (iqr 8,4 anni). I casi portatori di un difetto di tipo alfa talassemico sono risultati 83; 78 delezioni o mutazioni degli alfa geni e 5 portatori di triplicazione del gene che non sono stati inclusi nell'analisi dei cut-off (Vedi grafici a lato). L'MCV era inferiore a 80 fl in 29 casi (37,2%) e 60 avevano un MCH < 27 pg (76,9%). La sensibilità e la specificità, applicando uno dei due parametri, sono rispettivamente per l'MCV: 37,2% (intervallo di confidenza al 95%, IC, 26,5% - 48,9%) e 98,4% (IC 96,3% - 99,5%); per MCH: 76,9% (IC 66% - 85,7%) e 89,2% (IC 85,3% - 92,4%). Le curve ROC mostrano un valore di area sotto la curva (AUC) di 0,678 per l'MCV e di 0,831 per l'MCH (p<0,001).







Il valore massimo di AUC di MCV e di MCH per l'identificazione di un difetto alfa è risultato essere rispettivamente di 84,25 fl e di 27,55 pg, con un' AUC di 0,82 per l'MCV e di 0,84 per l'MCH (utilizzando sia lo Youden index che il metodo di Liu).

Discussione: le soglie identificate per il nostro laboratorio si discostano dalle linee guida internazionali per l'MCV, risultando essere più sensibile e specifico il valore di 84 fl mentre l'MCH < 27 pg si conferma essere un valore adeguato. In teoria il nostro risultato potrebbe avere diverse spiegazioni. Possiamo escludere bias di rilevamento, data l'alta affidabilità dei counter di ultima generazione. Abbiamo posto la massima cura nel prevenire bias di selezione, molto comuni in questo tipo di studi, escludendo tutti i soggetti che potessero essere stati studiati in base agli indici globulari. Limitando lo studio alla nostra attività per le coppie abbiamo voluto indirettamente, ma efficacemente limitare il bias per età, non includendo quindi la ben nota variabilità di MCV/MCH dei soggetti pediatrici (ed in parte degli anziani). Alcuni degli studi che supportano le linee guida includono soggetti in età pediatrica. Di nuovo diversi studi di riferimento per le linee guida sono stati fatti su popolazioni in cui la prevalenza di difetti alfa è maggiore, con indici globulari mediamente più bassi.

In conclusione, i nostri dati confermano l'utilità di individuare i propri valori di riferimento per migliorare la propria accuratezza diagnostica.

Incidenze dei difetti alfa analizzati nello studio

Mutazione	n	%
Eterozigote -3,7	52	66,7
Eterozigote α2 init cd (T>C)	5	6,4
- - MED	1	1,3
cluster α -25kb	1	1,3
Omozigote -3,7/-3,7	9	11,5
Eterozigote IVSI (-5nt)	5	6,4
-4,2	1	1,3
-20,5	2	2,6
Omozigote α2 init cd (T>C)	1	1,3
Eterozigote c.91G>A	1	1,3
TOTALE	78	

MCH<27

Standardizzazione dei valori di laboratorio per la diagnosi di portatore di alfa talassemia

Vincenzo Voi[1], Lidia Cereda[1], Emanuele Pivetta[2], Antonio Piga[1]

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[2] Epidemiologia dei tumori e CRPT U, Medicina d'Urgenza – MECAU, Dipartimento Scienze Mediche, Università di Torino, AOU Città della Salute e della Scienza di Torino – Presidio Molinette

Introduzione: La maggior parte delle linee guida ritengono un MCV < 79 fl e un MCH < 27 pg utili allo screening diagnostico di portatore sano di alfa talasser

Obiettivo dello Studio: verifi valori soglia di MCV e MCH prevenzione.

Metodi: abbiamo incluso nel beta talassemia e HbS afferen

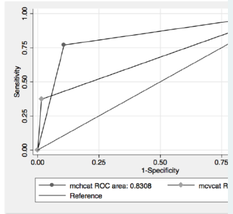
La diagnosi molecolare è stat

Criteri di esclusione: soggetti con associazione di) di altra e 30 ng/dl; soggetti con patolo

È stata calcolata l'accuratezza

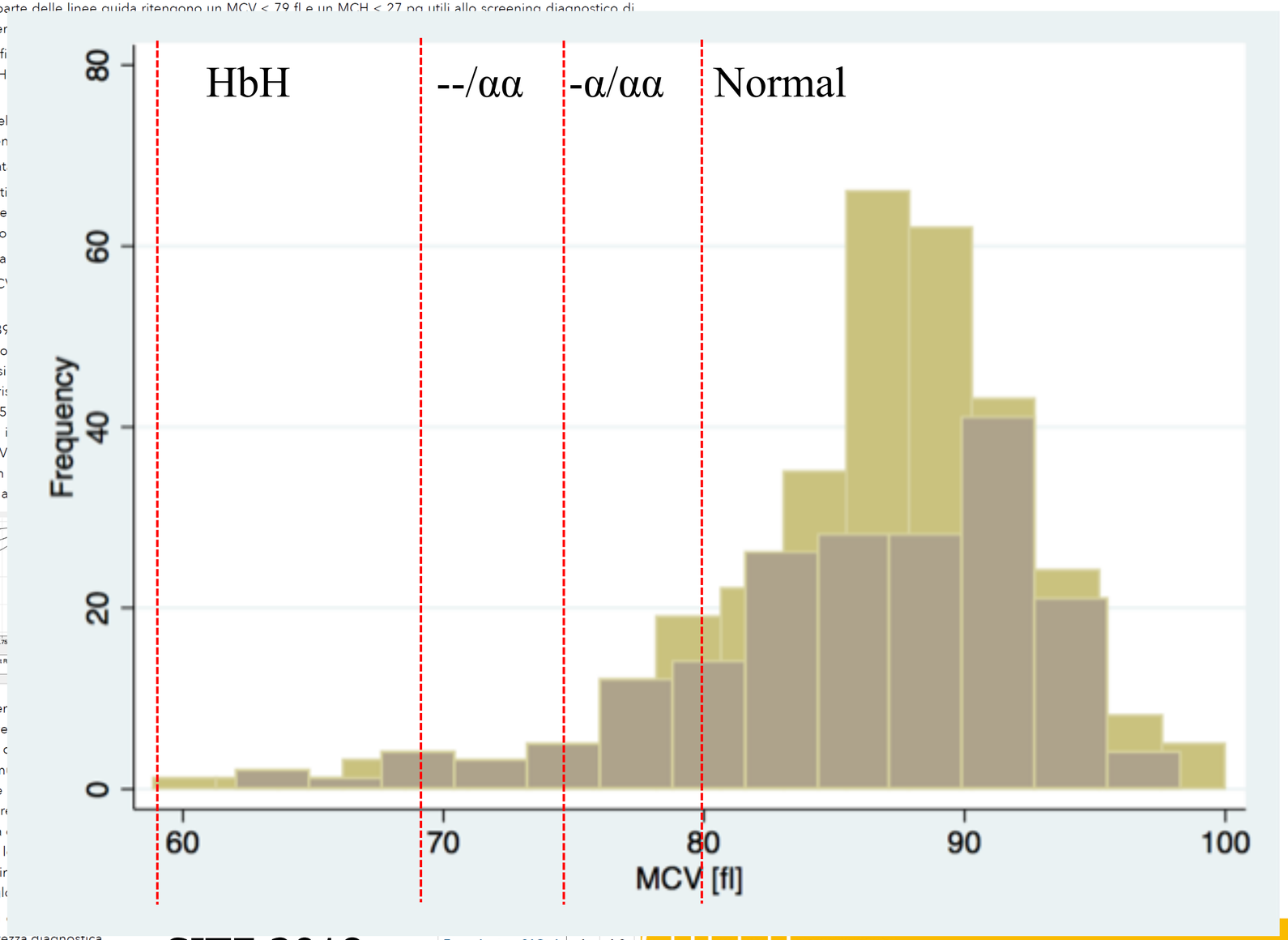
Per individuare i valori di MC il metodo di Liu).

Risultati: da un totale di 489 state incluse 394 osservazio 33,5 anni (iqr 8,4 anni). I casi tipo alfa talassemico sono ris mutazioni degli alfa geni e 5 del gene che non sono stati i off (Vedi grafici a lato). L'MCV casi (37,2%) e 60 avevano un sensibilità e la specificità, a



specifico il valore di 84 fl mer nostro risultato potrebbe ave l'alta affidabilità dei counter c bias di selezione, molto com essere stati studiati in base coppie abbiamo voluto indir quindi la ben nota variabilità degli studi che supportano l studi di riferimento per le lir alfa è maggiore, con indici gl

In conclusione, i nostri dati migliorare la propria accuratezza diagnostica.



Eterozigote c.91G>A	1	1,3
TOTALE	78	

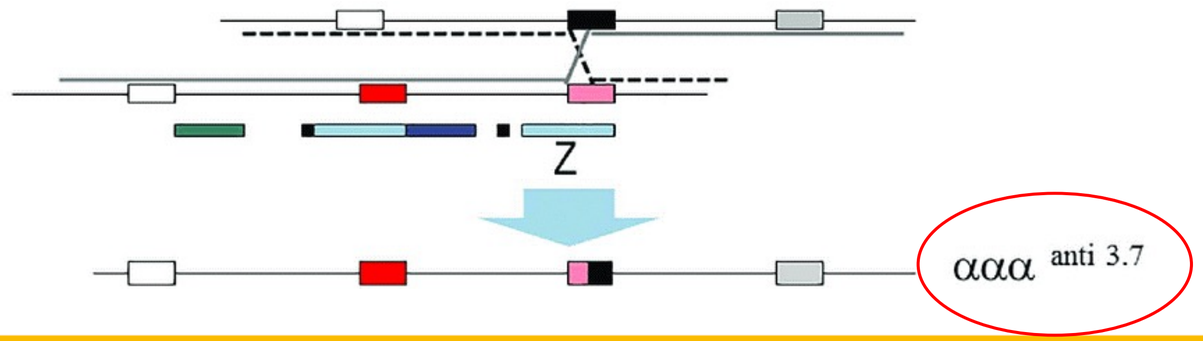
Alpha thalassemia carrier

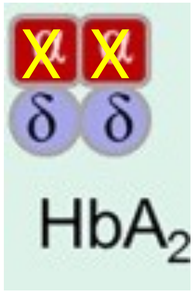
12 – 15 g/dl

13 – 17 g/dl

MCV ≤ 80 fl

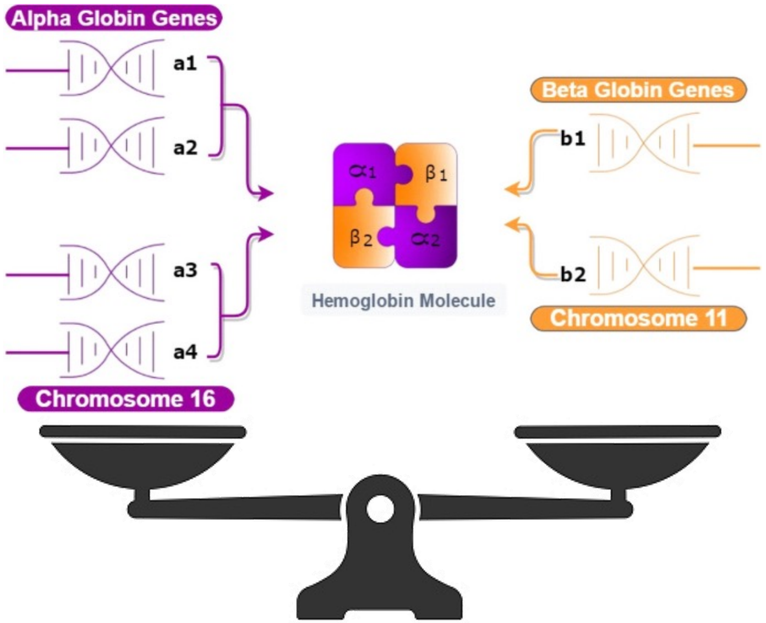
MCH <27 pg

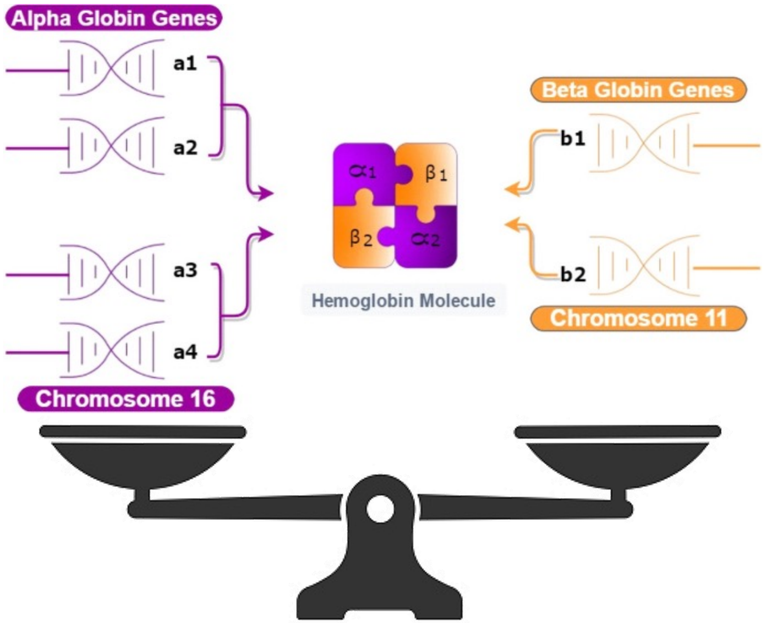




2 – 3,2 %

0 – 1,5 %


Alpha Globin Genes
a1
a2
a3
a4
Chromosome 16
α1
β1
β2
α2
Hemoglobin Molecule
Beta Globin Genes
b1
b2
Chromosome 11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b1
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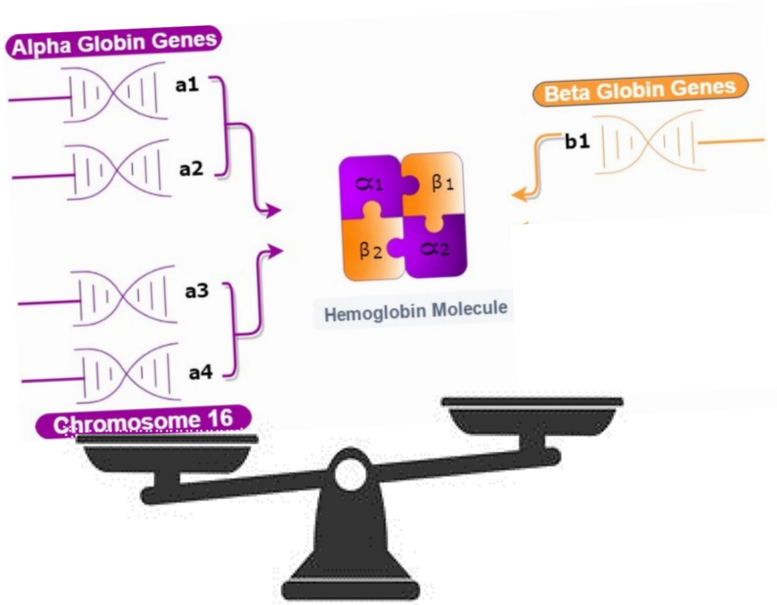
Chromosome 11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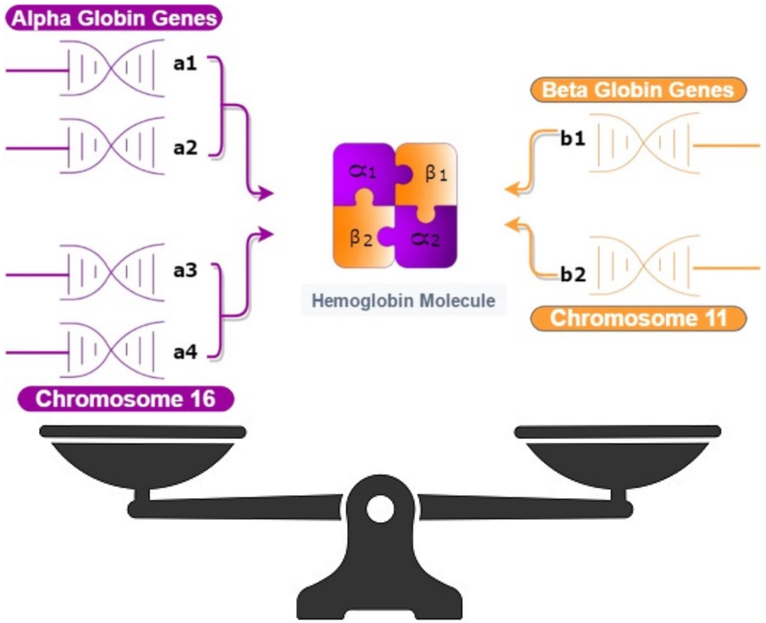
a1
a2
a3
a4
Chromosome 16
Hemoglobin Molecule
Beta Globin Genes
b1


Alpha Globin Genes
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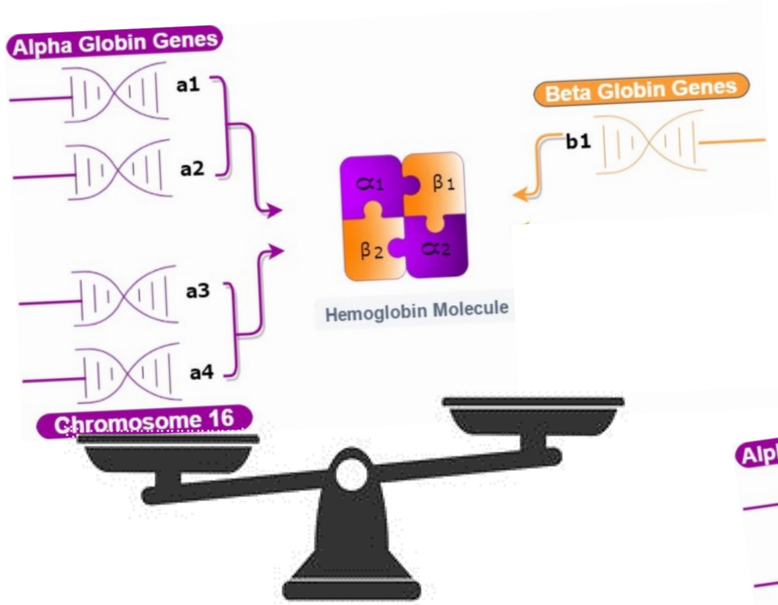
Chromosome 16
Hemoglobin Molecule
Beta Globin Genes
b1
b2
Chromosome 11


Alpha Globin Genes
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a2
a3
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Hemoglobin Molecule
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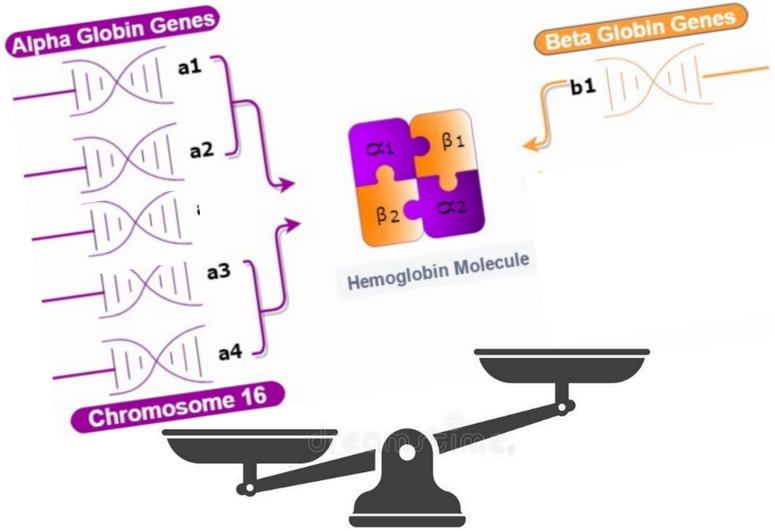
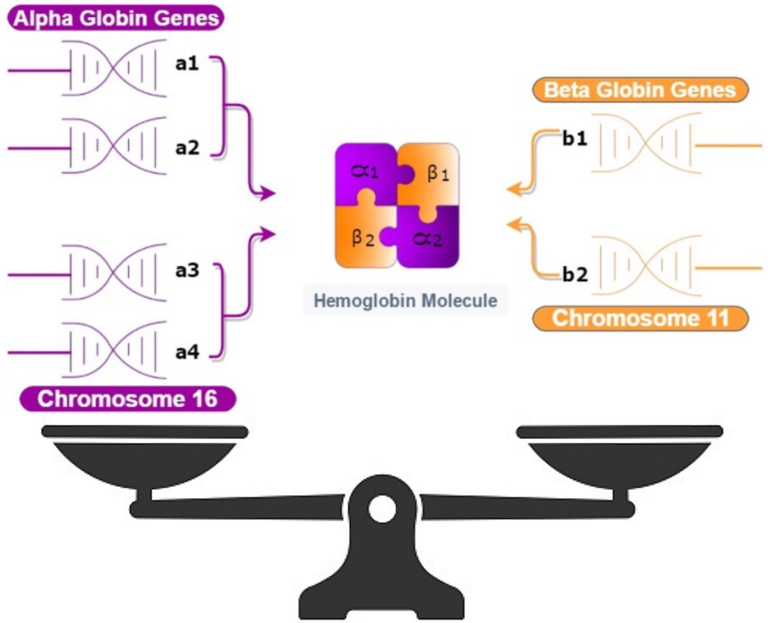
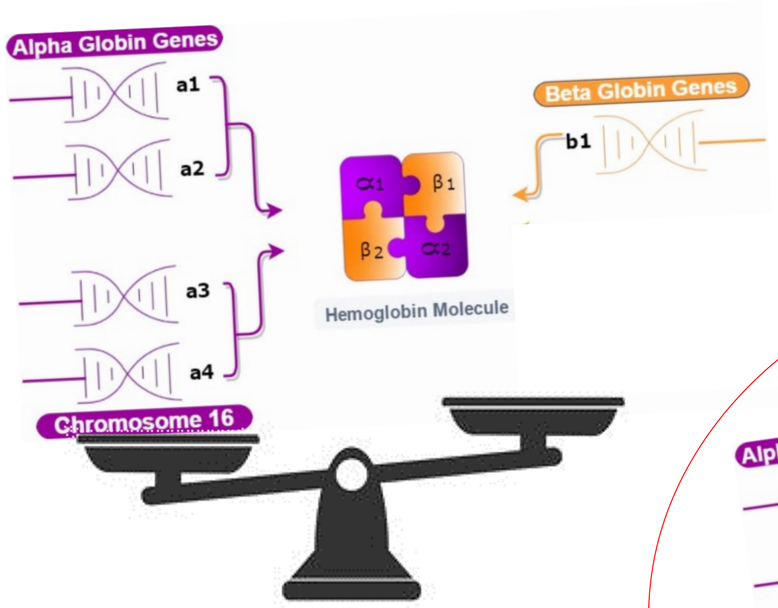
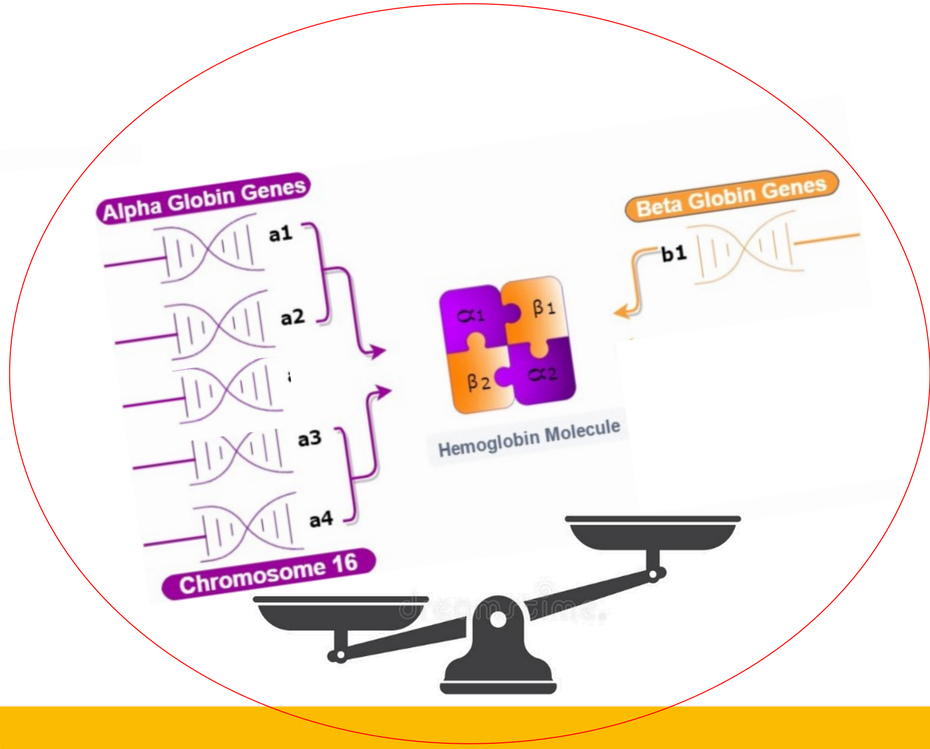
b1


Alpha Globin Genes
a1
a2
a3
a4
Chromosome 16
Hemoglobin Molecule
Beta Globin Genes
b1





Thal Intermedia - NTDT



Hb	14,8
MCV	81,7
MCH	26,9
RDW	14,7
Serum Iron	64
Transferrin	132
Transferrin sat	20%
Ferritin	224
HbA2	2,6
HbF	0,6

Female, pregnant

Hb	9,6
MCV	61,7
MCH	19,7
RDW	11,9
Serum Iron	33
Transferrin	198
Transferrin sat	11%
Ferritin	38
HbA2	5,3
HbF	1,0

Female, pregnant

Hb	11,9
MCV	92,1
MCH	30,3
RDW	13,4
Serum Iron	69
Transferrin	263
Transferrin sat	18%
Ferritin	63
HbA2	3,6
HbF	1,2

6 months, male

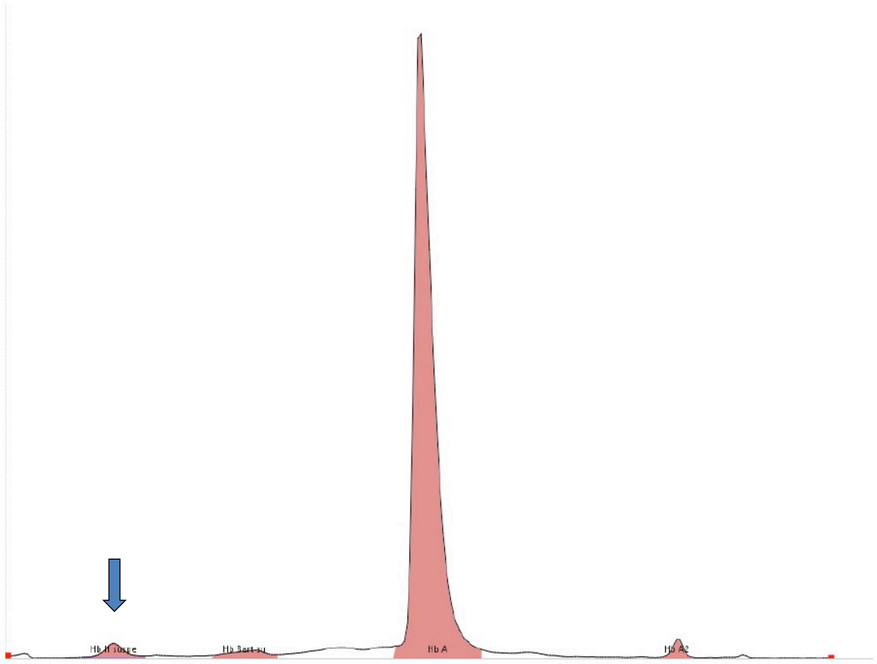
Anemization during infection

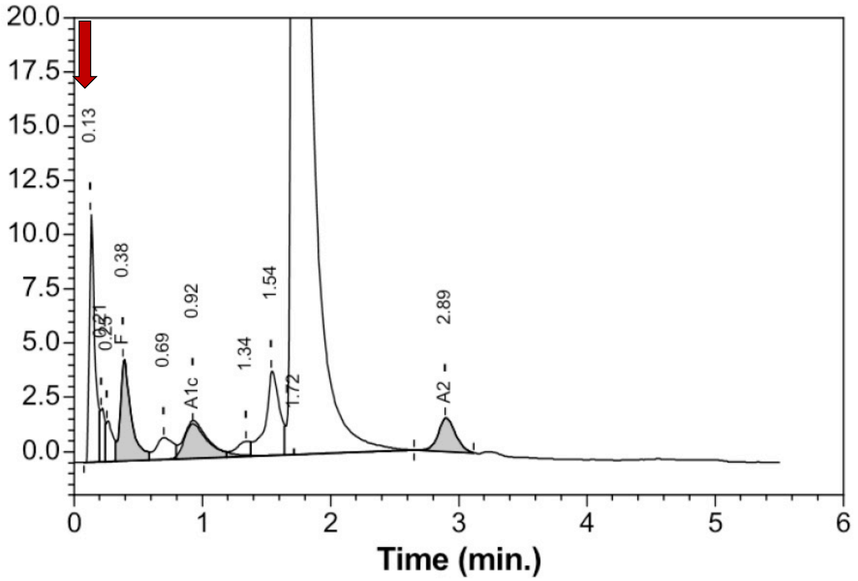
Hb	8,2	11-14
MCV	56,8	68-84
MCH	18,9	24-30
RDW	12,7	12-15
Serum Iron	53	
Transferrin	210	
Transferrin sat	17%	
Ferritin	45	
HbA2	4,4	
HbF	13,4	

Hb	10
MCV	57,6
MCH	17,4
RDW	11,3

Serum Iron	121
Transferrin	214
Transferrin sat	39%
Ferritin	727

HbA2	1,6
HbF	3,1
?	3,7



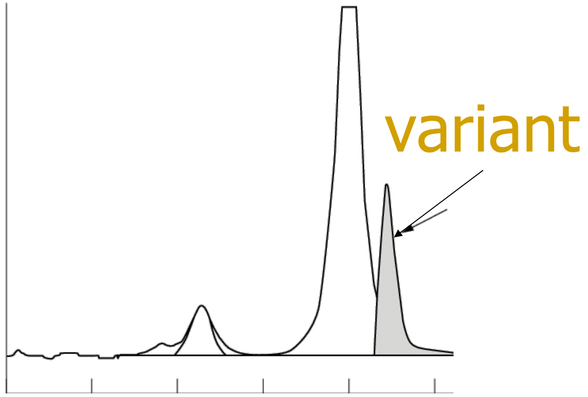


Hb	12,7
MCV	87,5
MCH	28,8
RDW	12,7
Serum Iron	103
Transferrin	241
Transferrin sat	30%
Ferritin	66
HbA2	2,2
HbF	7,6

Hb	14,6
MCV	87,5
MCH	28,3
RDW	12,9
Serum Iron	104
Transferrin	275
Transferrin sat	11%
Ferritin	106
HbA2	1,5
HbF	0,5
HbX	1,1

Variant haemoglobin

- Stability
- O_2 affinity



α 4 genes → 25%

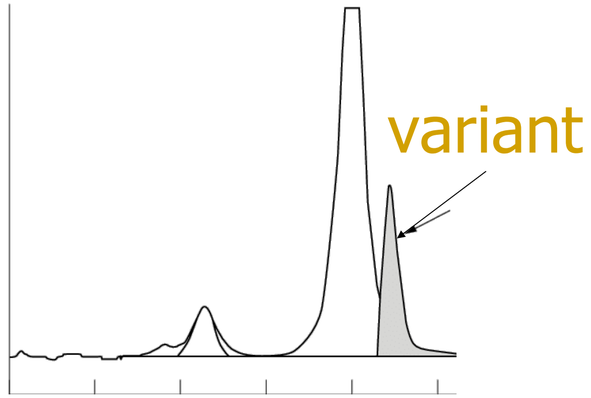
β 2 genes → 50%

δ 2 genes → 50%

Variant haemoglobin

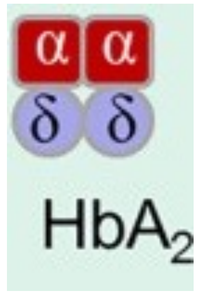
1[illegible] – 15 g/dl

13 – 17 g/dl

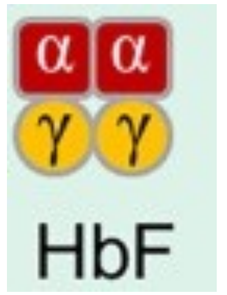


MCV 80 – 1[illegible]0 fl

MCH 27 – 31 pg



2 – [illegible],2 %



0 – [illegible],5 %

p50[illegible]

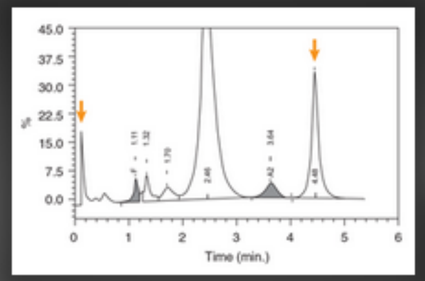
https://hemoglobins.bio-rad.com/Pages/ChromatogramPage.aspx?labid=15

BIO-RAD

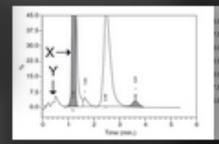
Information | Library of Variants | Founding Contributors | Bio-Rad Support Centers | Institutional Resources

1 / 17

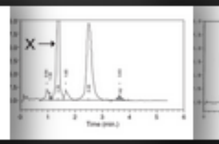
Double-click for more information



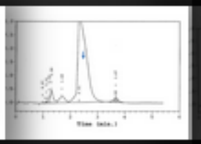
H Disease and HbS, 0.15



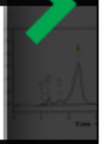
J Iran, 1.16



Hope, 1.35



San Diego, 2.31



HbS/La De

2.3

Case Type

Educational References

Platform

VARIANT™ II

Retention time

Hb name

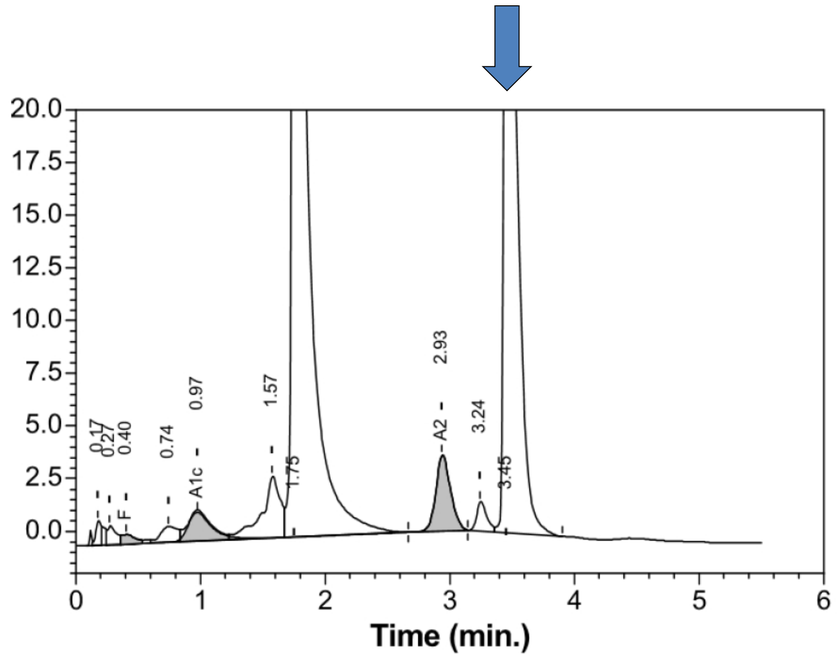
Hb classes

Frequency / Occurence

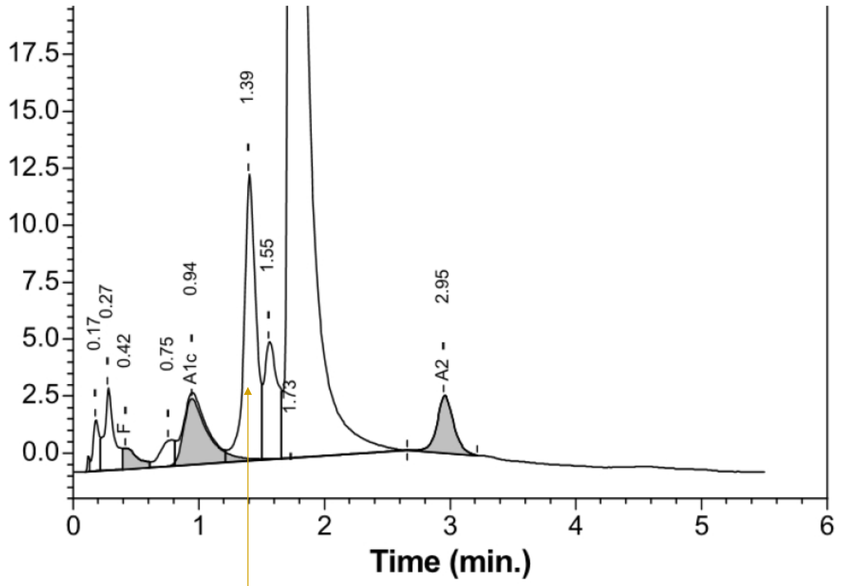
Search

Reset

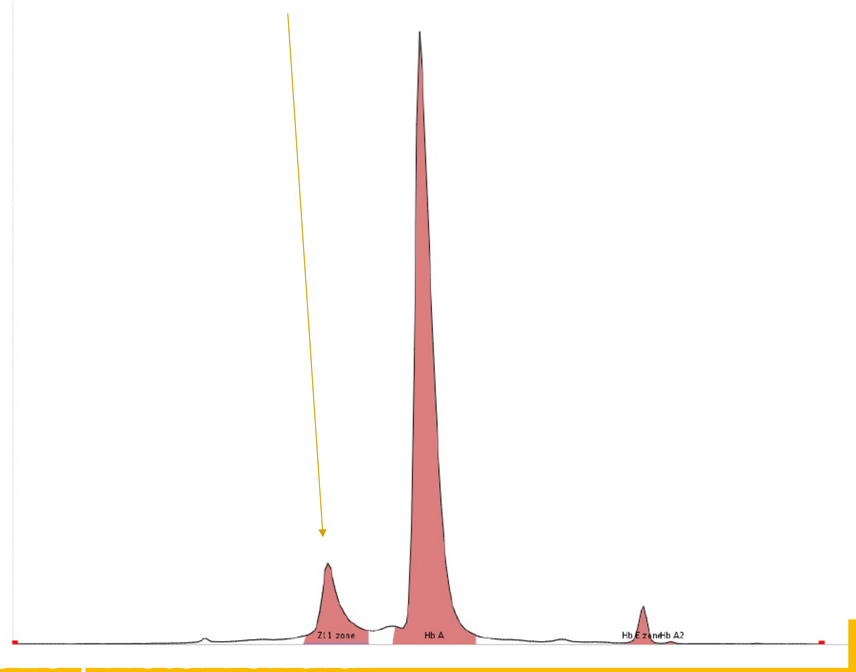
Hb	15,5
MCV	83,3
MCH	26,4
RDW	14
Serum Iron	103
Transferrin	181
Transferrin sat	40%
Ferritin	345
HbA2	3,6
HbF	0,1
HbS	33,4



Hb	16,6
MCV	89,3
MCH	27,7
RDW	13,1
Serum Iron	104
Transferrin	280
Transferrin sat	26%
Ferritin	124
HbA2	2,5
HbF	0,56
HbX	9,1



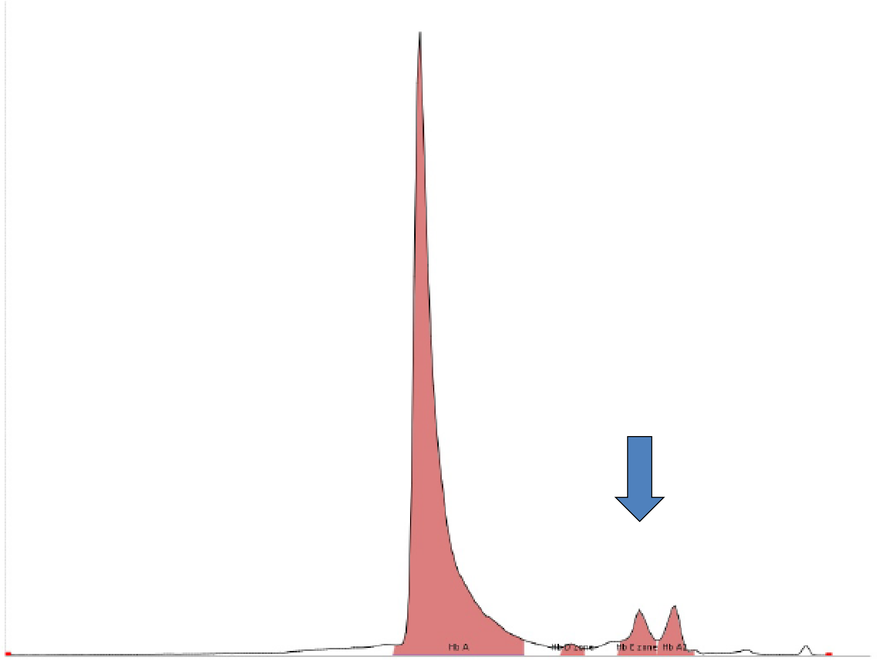
Variant ?

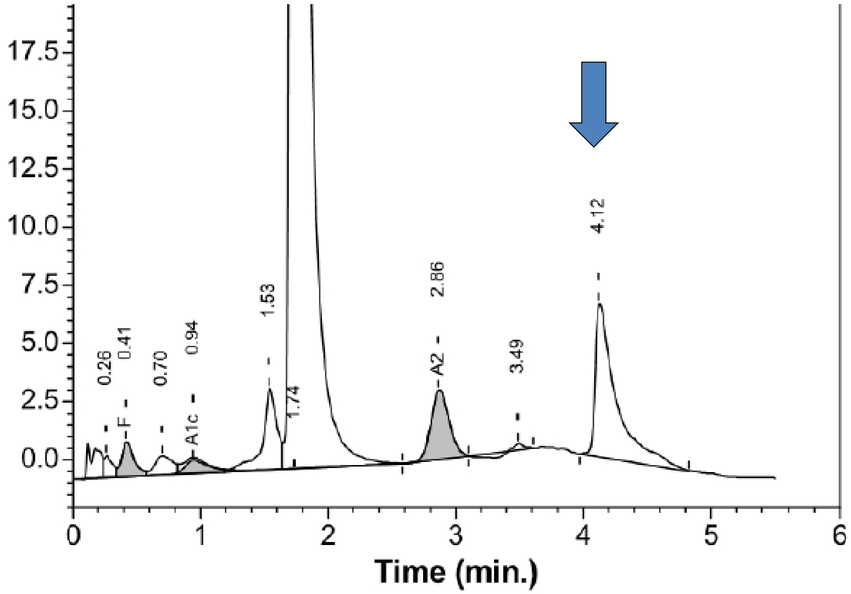


Hb	11,1
MCV	94,2
MCH	26,6
RDW	19,4

Serum Iron	87
Transferrin	207
Transferrin sat	30%
Ferritin	10

HbA2	3
HbF	0,8
HbX	7,7





Molecular analysis is a second level test

Diagnostica di I e II livello delle Emoglobinopatie

Buone Pratiche SITE

European Journal of Human Genetics (2015) 23, 426–437

www.nature.com/ejhg

EJHG Open

POLICY

EMQN Best Practice Guidelines for molecular and haematology methods for carrier identification and prenatal diagnosis of the haemoglobinopathies

SITE SOCIETA' ITALIANA TALASSEMIE ED EMOGLOBINOPATIE

- First level test not conclusive
  - variant Hb characterization
  - α talassemia carrier (α+)
  - β+/silent mutations (e.g. borderline HbA2)

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  - variant Hb characterization
  - α talassemia carrier (α+)
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- «double carrier»: suspicion of α and β thalassemia carrier, in order to correctly define the recurrence risk

- First level test not conclusive
  - variant Hb characterization
  - α talassemia carrier (α+)
  - β+/silent mutations (e.g. borderline HbA2)

- «double carrier»: suspicion of α and β thalassemia carrier, in order to correctly define the recurrence risk

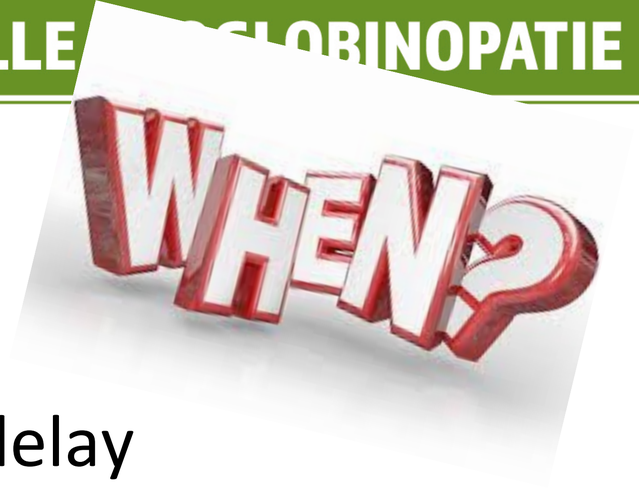
- To define the mutation in a carrier (gen-phen correlation)

Table 5 Genetic variations associated with normal/borderline Hb A2 levels—a guideline of related haematological and biosynthetic characteristics

Variation HGVS nomenclature NM_000518.4 (HBB)	*Variation traditional nomenclature*	*MCV fl*	*MCH pg*	*Hb A_2*	*α/β ratio*
c.−151C>T	β −101 (C→T)	88.5 ± 7.8	30.1 ± 1.0	3.1 ± 1.0	1.3 ± 0.4
c.−142C>T	β −92 (C(T)	83.0 ± 6.0	28.3 ± 2.0	3.5 ± 0.4	1.3 ± 0.8
c.−18C>G	β +33 (C(G)	82.0 ± 9.2	27.1 ± 3.4	2.5 ± 1.4	1.3 ± 0.6
c.316-7C>G	βIVS2-844 (C→G)	96.0 ± 4.0	30.3 ± 1.8	3.2 ± 0.2	1.0 ± 0.6
c.*6C>G	β +1480 (C→G)	88.3 ± 9.5	27.9 ± 6.0	2.7 ± 0.8	1.6 ± 0.4
	$\alpha\alpha\alpha/\alpha\alpha$	85.5 ± 7.8	30.4 ± 5.0	2.8 ± 0.6	1.2 ± 0.4
	KLF1 variants (29)	82.7 ± 5.7	27.8 ± 2.2	3.6 ± 0.2	
c.−50A>C	Cap + 1 (A(C)	23–26*	75–80*	3.4–3.8*	—
c.92 + 6T>C	β IVS1-6 (T→C)	71.0 ± 4.0	23.1 ± 2.2	3.4 ± 0.2	1.9 ± 1.0
	$\delta + \beta$ thalassaemia	64.3 ± 4.0	20.9 ± 1.4	3.6 ± 0.2	1.7 ± 0.6

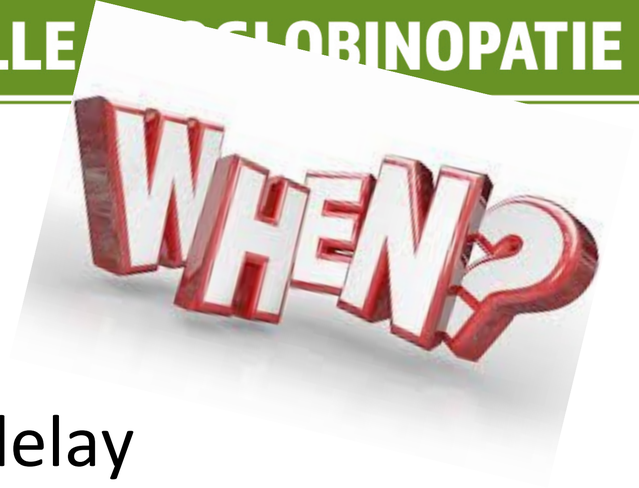
Values (mean ± 2SD or range (*)) are a guideline and represent those reported in various studies on carriers of these variants (prepared by R Galanello).
Note: It is recommended that subjects with borderline Hb A2 levels, particularly if their partner is a typical β-thalassaemia carrier, should be extensively investigated (α and β gene analysis, globin biosynthesis), although the majority usually have normal HBB and HBA genes. Borderline-raised Hb A2 levels in normal individuals are usually explained as the extreme distribution of the normal range of the Hb A2.
Furthermore, in couples where one partner is heterozygous for a severe α-thalassaemia defect and the other is a β-thalassaemia carrier, it is recommended that the *HBA* gene cluster be fully characterized in the β-thalassaemia carrier in order to preclude any risk of offspring with severe Hb H disease or Hb Bart's hydrops.

Traeger-Synodinos J et al. European Journal of Human Genetics (2015) 23



» Sideropenia in pregnant couples:

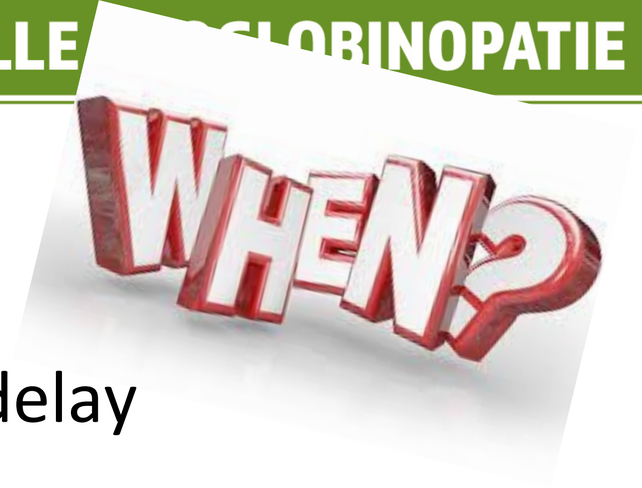
α and β globin genes analysis if the partner is a carrier in order to avoid PND delay



» Sideropenia in pregnant couples:

α and β globin genes analysis if the partner is a carrier in order to avoid PND delay

» Exclude alpha globin gene triplication/quadruplication in the partner of a β thalassemia carrier to rule out NTDT risk



» Sideropenia in pregnant couples:

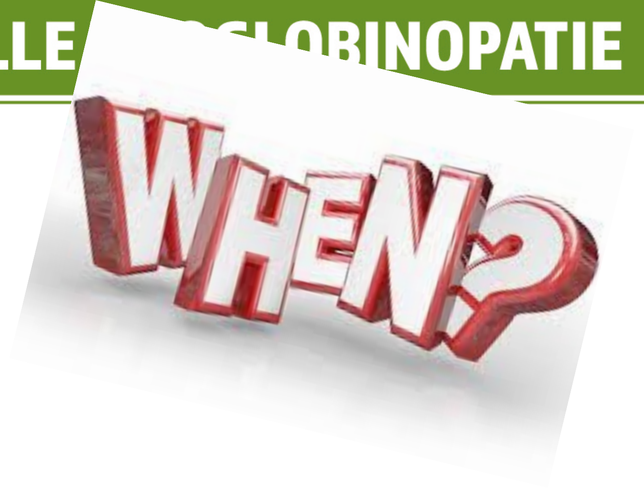
α and β globin genes analysis if the

partner is a carrier in order to avoid PND delay

» Exclude alpha globin gene triplication/quadruplication in the partner of a β thalassemia carrier to rule out NTDT risk

» Before prenatal diagnosis:

- Parental DNA must be known
- PND 50 is possible but residual risk

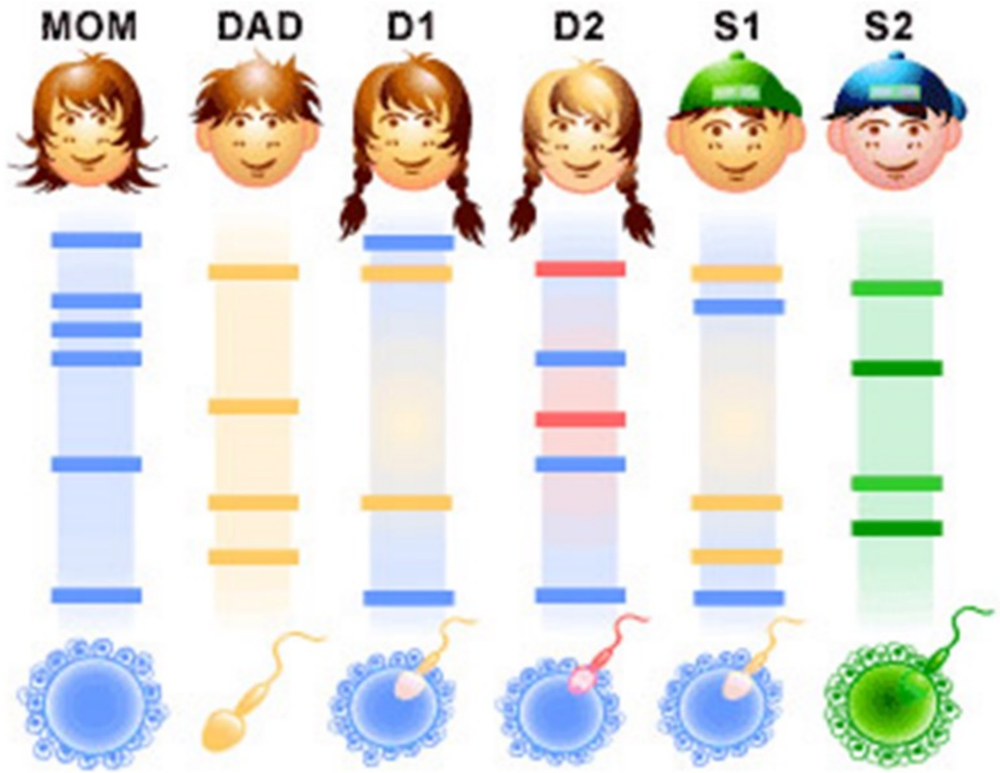


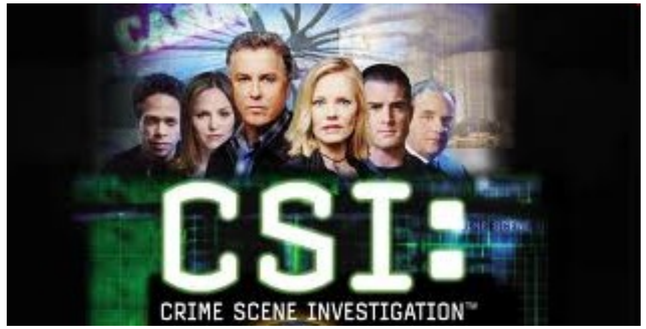
» Before prenatal diagnosis:
  – Parental DNA must be known
  – PND 50 is possible but residual risk

(2 different techniques – maternal cell contamination)

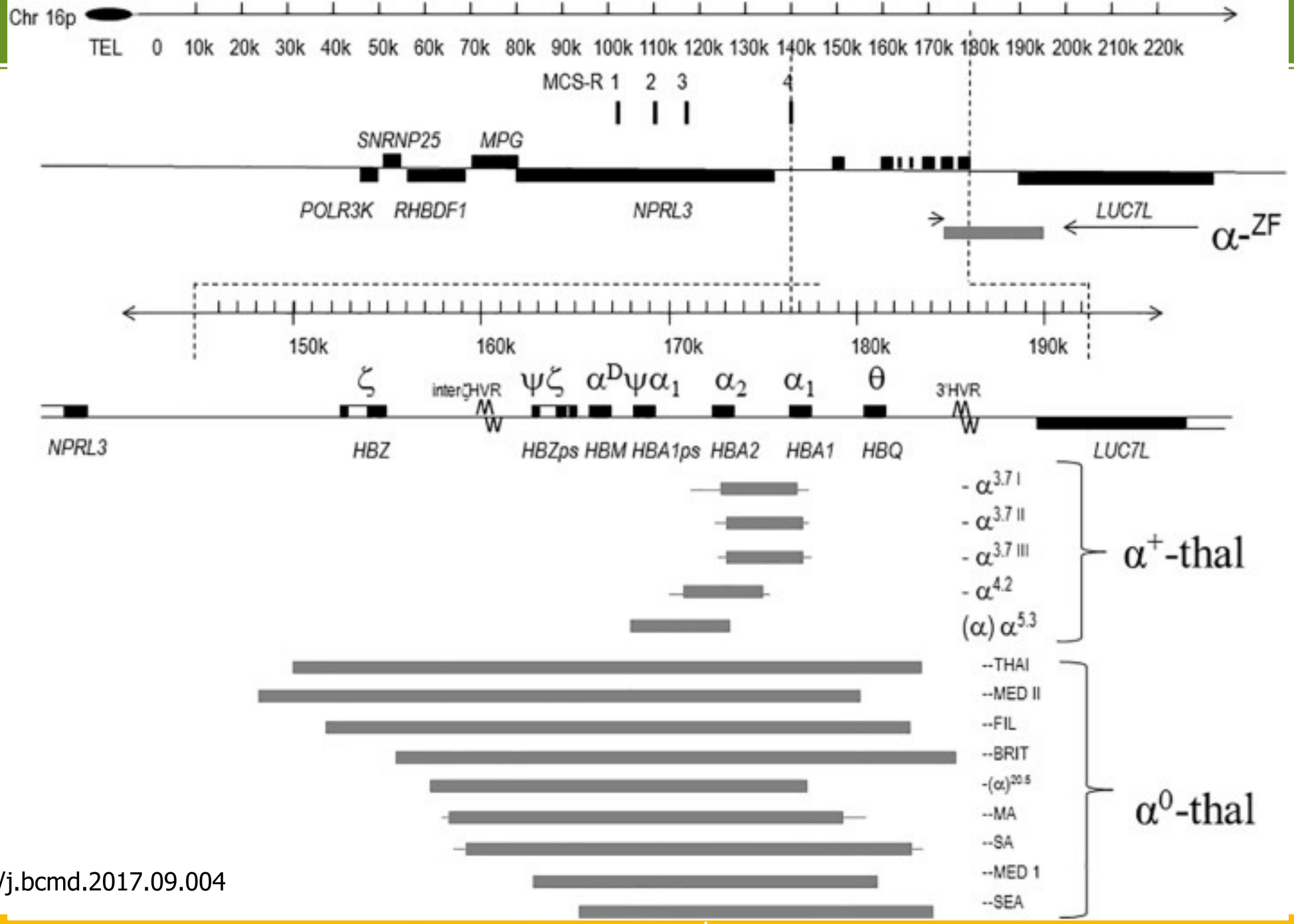
Paternity – maternal contamination

Microsatellite= short repeated DNA sequences, non coding, highly polymorphic



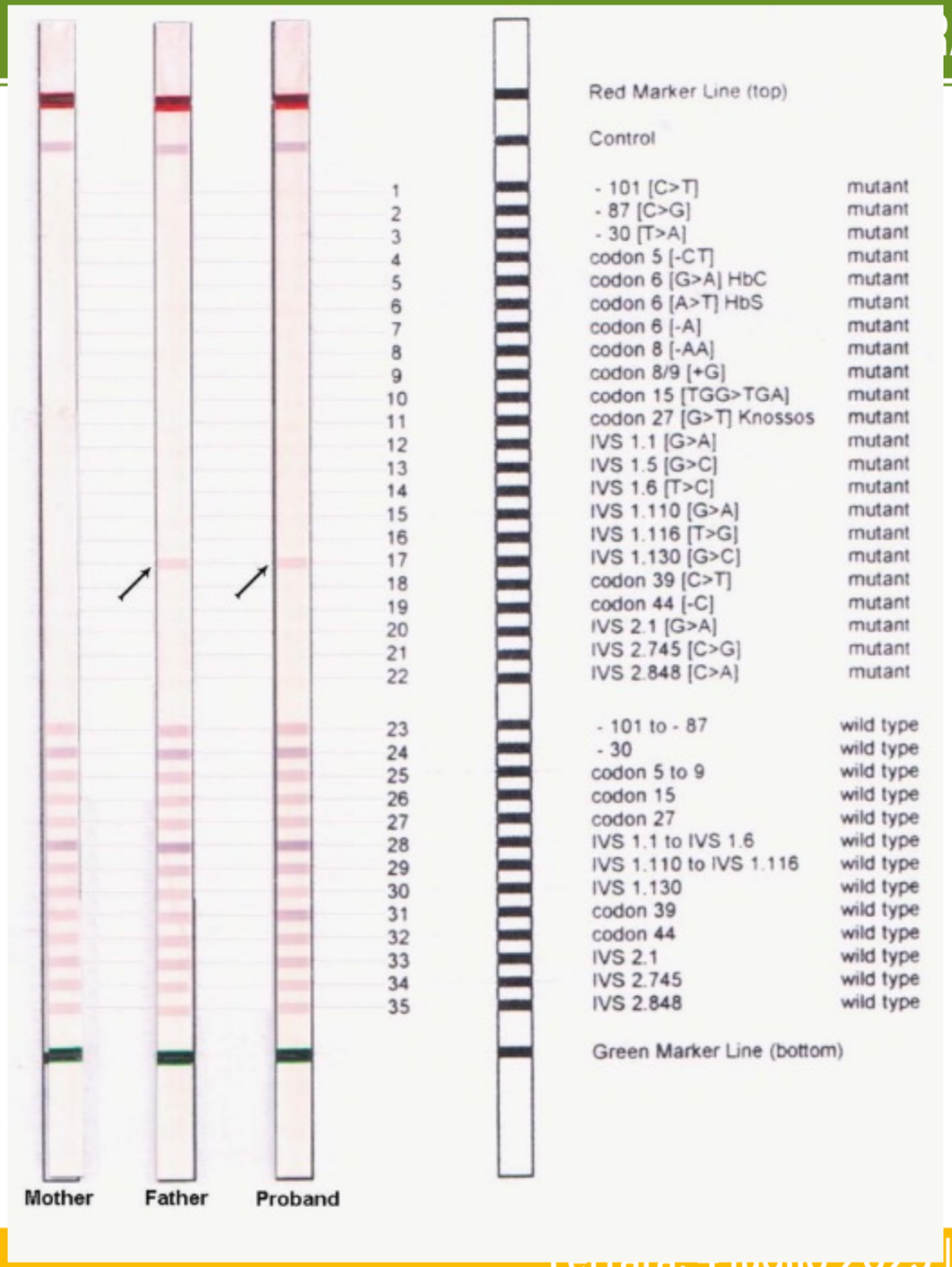


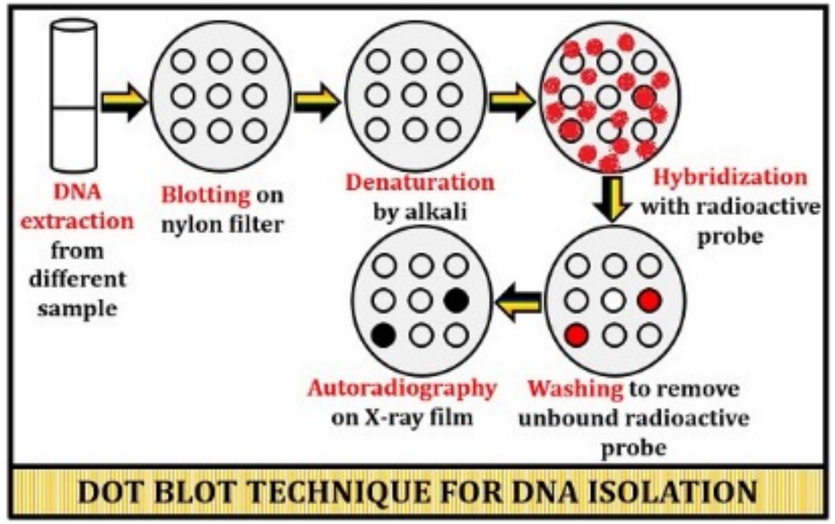
- α-globin genes more prone to deletion/duplication



doi.org/10.1016/j.bcmd.2017.09.004

- α-globin genes more prone to deletion/duplication
- β-globin gene more prone to point mutations


Red Marker Line (top)
Control
1 - 101 [C>T] mutant
2 - 87 [C>G] mutant
3 - 30 [T>A] mutant
4 codon 5 [-CT] mutant
5 codon 6 [G>A] HbC mutant
6 codon 6 [A>T] HbS mutant
7 codon 6 [-A] mutant
8 codon 8 [-AA] mutant
9 codon 8/9 [+G] mutant
10 codon 15 [TGG>TGA] mutant
11 codon 27 [G>T] Knossos mutant
12 IVS 1.1 [G>A] mutant
13 IVS 1.5 [G>C] mutant
14 IVS 1.6 [T>C] mutant
15 IVS 1.110 [G>A] mutant
16 IVS 1.116 [T>G] mutant
17 IVS 1.130 [G>C] mutant
18 codon 39 [C>T] mutant
19 codon 44 [-C] mutant
20 IVS 2.1 [G>A] mutant
21 IVS 2.745 [C>G] mutant
22 IVS 2.848 [C>A] mutant
23 - 101 to - 87 wild type
24 - 30 wild type
25 codon 5 to 9 wild type
26 codon 15 wild type
27 codon 27 wild type
28 IVS 1.1 to IVS 1.6 wild type
29 IVS 1.110 to IVS 1.116 wild type
30 IVS 1.130 wild type
31 codon 39 wild type
32 codon 44 wild type
33 IVS 2.1 wild type
34 IVS 2.745 wild type
35 IVS 2.848 wild type
Green Marker Line (bottom)
Mother
Father
Proband

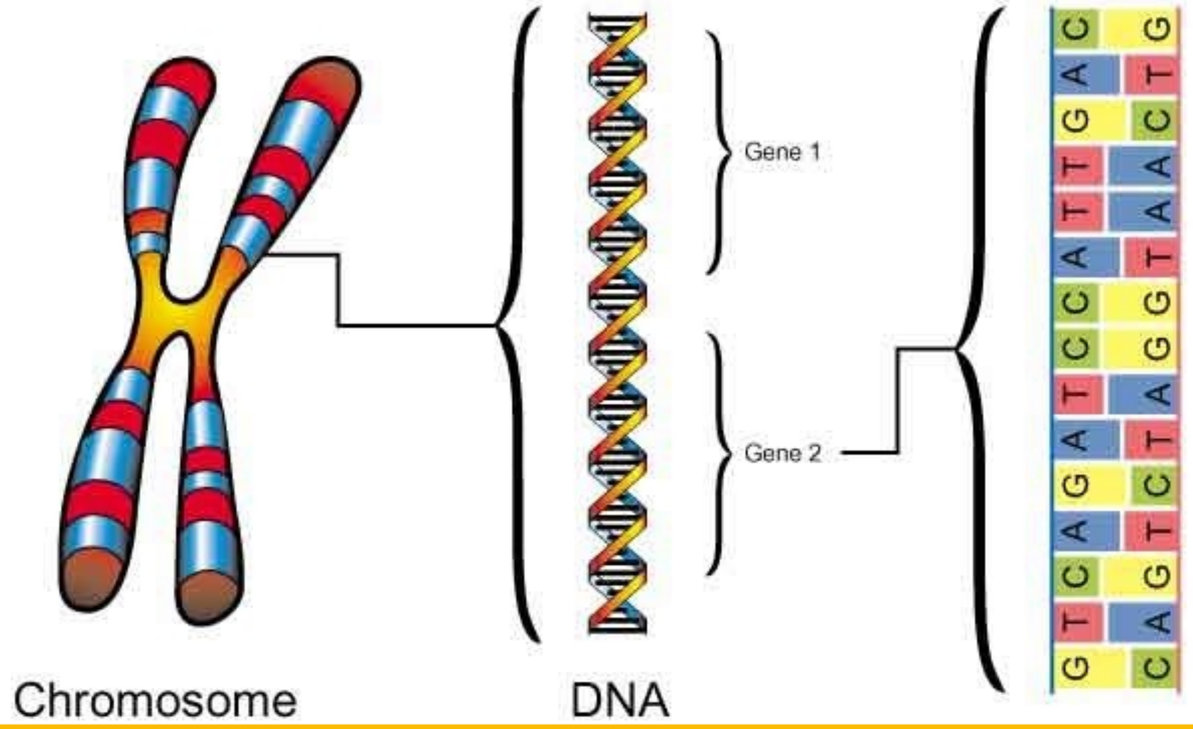

DNA extraction from different sample
Blotting on nylon filter
Denaturation by alkali
Hybridization with radioactive probe
Washing to remove unbound radioactive probe
Autoradiography on X-ray film
DOT BLOT TECHNIQUE FOR DNA ISOLATION

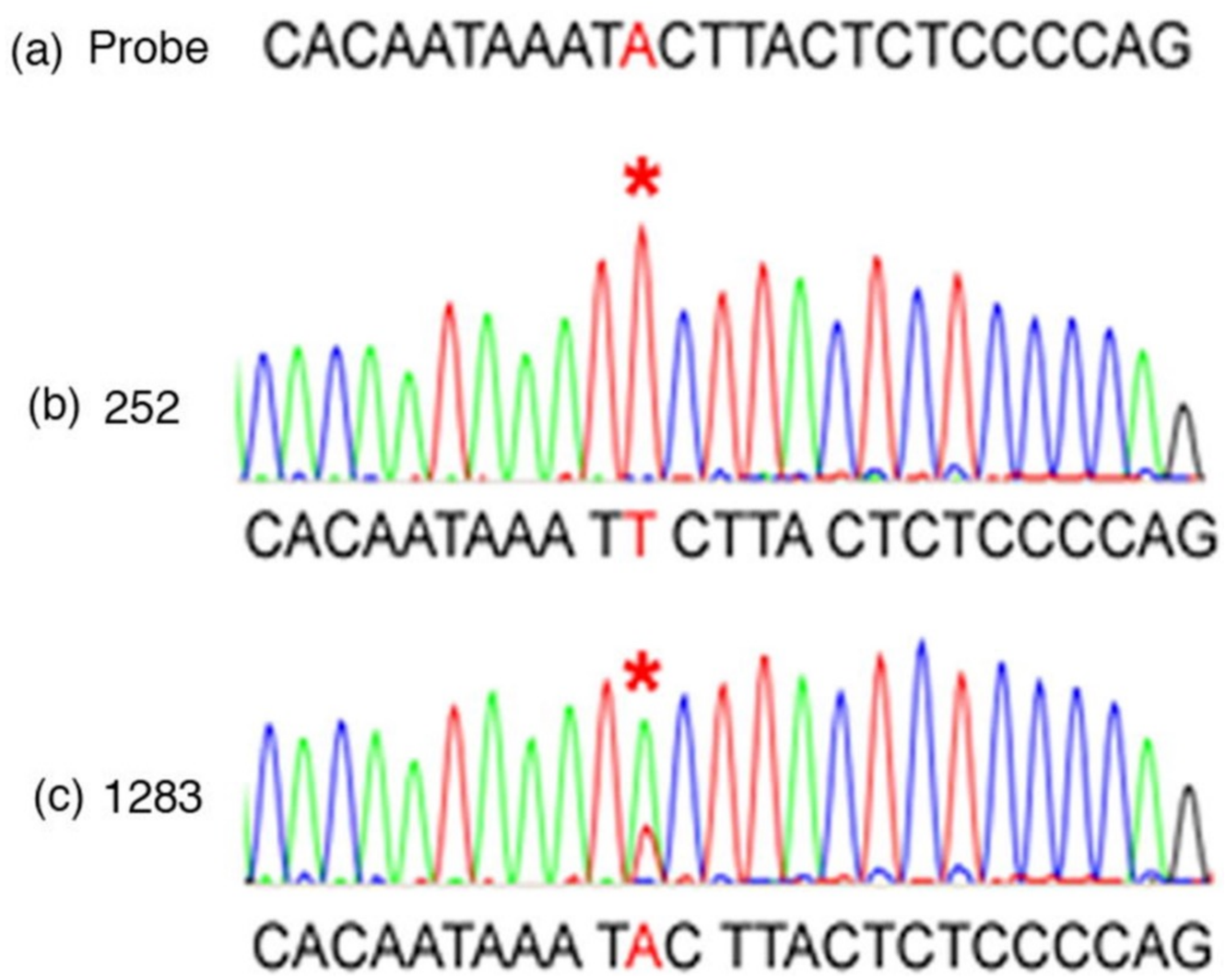
Single gene analysis

» DNA extraction

» PCR

» Sequencing




(a) Probe CACAATAAATACTTACTCTCCCCAG
(b) 252
CACAATAAA TT CTTA CTCTCCCCAG
(c) 1283
CACAATAAA TAC TTACTCTCCCCAG

Next generation sequencing

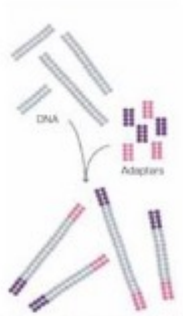


Figure 1
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

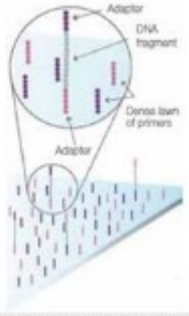


Figure 2
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

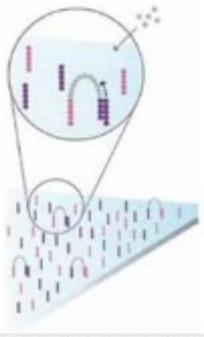


Figure 3
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

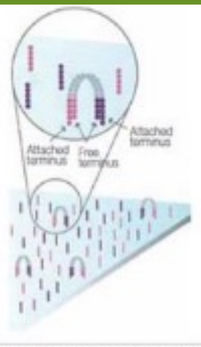


Figure 4
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

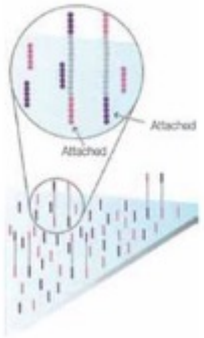


Figure 5
Denaturation leaves single-stranded templates anchored to the substrate.

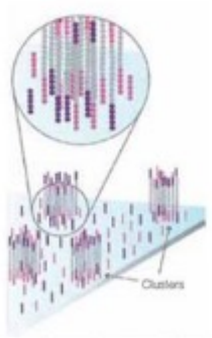


Figure 6
Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

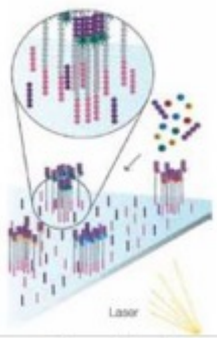


Figure 7
The first sequencing cycle begins by adding four labeled reversible terminators, primers, and DNA polymerase.

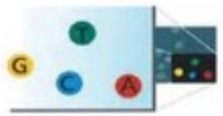


Figure 8
After laser excitation, the emitted fluorescence from each cluster is captured and the first base is identified.

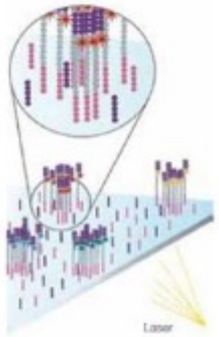


Figure 9
The next cycle repeats the incorporation of four labeled reversible terminators, primers, and DNA polymerase.

Figure 10
After laser excitation, the image is captured as before, and the identity of the second base is recorded.

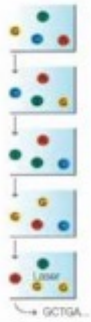


Figure 11
The sequencing cycles are repeated to determine the sequence of bases in a fragment, one base at a time.

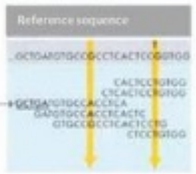
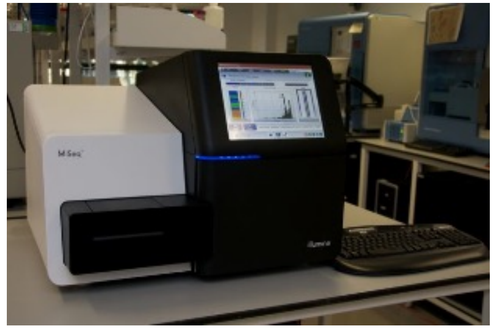


Figure 12
The data are aligned and compared to reference, and sequencing differences are identified.



genes

MDPI

Article

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Rita Selvatici [1,2,†], Valentina Guida [3,†], Massimo Maffei [4], Milena Agata Irrera [5], Alice Margutti [1,2], Paola Bisceglia [3], Massimo Mogni [4], Erica Melchionda [5], Giuseppina Stoico [1,2], Nicoletta Grifone [3], Laura Bocciardo [4], Simone Salerio [5], Vittoria Nagliati [1,2], Angela Alberico [3], Giusy Tringali [4], Cristina Melles [5], Alessandro De Luca [3], Alessandra Ferlini [1,2], Domenico Coviello [4,*] and Cristina Curcio [5]

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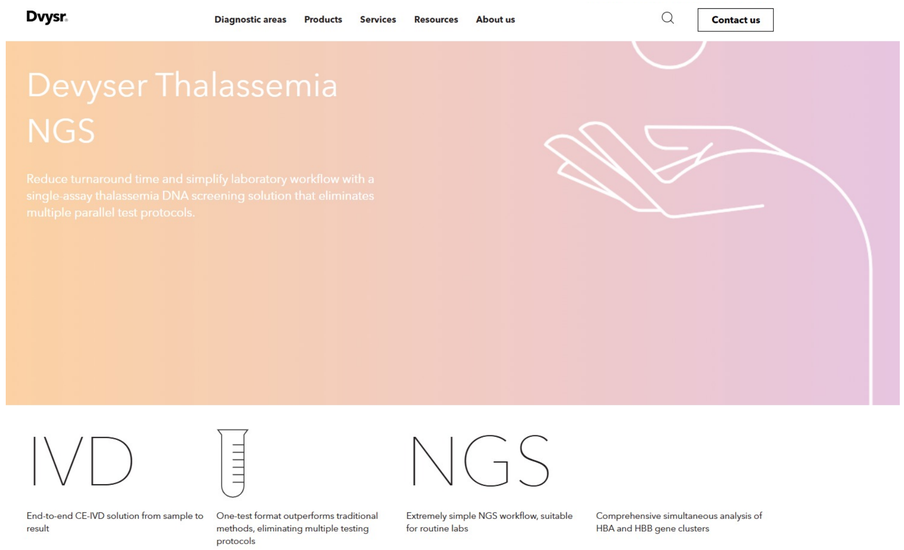

RDB missed around: 6.5% beta mutations

16% alfa mutations

5% missed diagnosis

Overall around 8,5% pts with rare variants

RDB 450 euros each - NGS 800 euros

genes

MDPI

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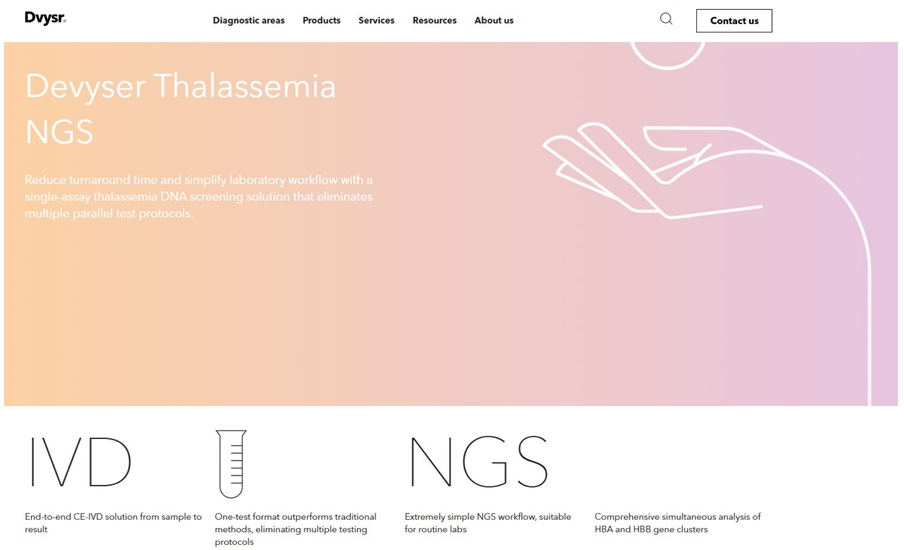


- high homology of the HBA1 HBA2 genes –no distinction of specific variants

- 1 case of false negative result, since the patient carried both the –3.7 deletion and the triple anti-α 3.7 in the α-globin locus

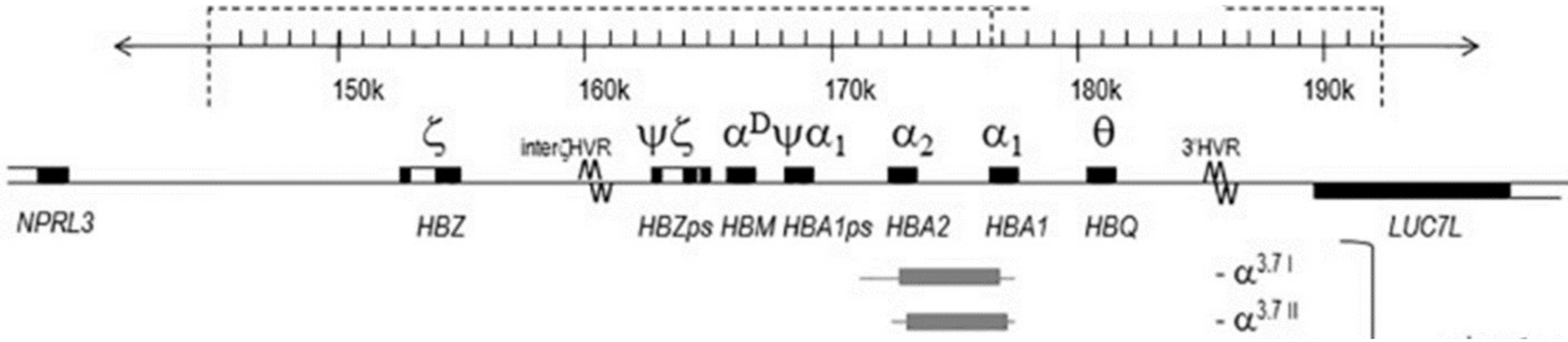
Multiple samples with deletions/duplications in the same session sometimes did not allow the algorithm to clearly call the most frequent CNVs - MLPA analysis.

Hb	14,8
MCV	81,7
MCH	26,9
RDW	14,7

Serum Iron	64
Transferrin	132
Transferrin sat	20%
Ferritin	224

HbA2	2,6
HbF	0,6

Alpha -3.7 het



Hb	9,6
MCV	61,7
MCH	19,7
RDW	11,9
Serum Iron	33
Transferrin	198
Transferrin sat	11%
Ferritin	38
HbA2	5,3
HbF	1,0

Female, pregnant

Alpha normal

Beta CD39 het

Hb	11,9
MCV	92,1
MCH	30,3
RDW	13,4
Serum Iron	69
Transferrin	263
Transferrin sat	18%
Ferritin	63
HbA2	3,6
HbF	1,2

Female, pregnant

Alpha normal

Beta -101 het

Hb	8,2	11-14
MCV	56,8	68-84
MCH	18,9	24-30
RDW	12,7	12-15
Serum Iron	53	
Transferrin	210	
Transferrin sat	17%	
Ferritin	45	
HbA2	4,4	
HbF	13,4	

6 months, male

Anemization during infection

Alpha anti3.7

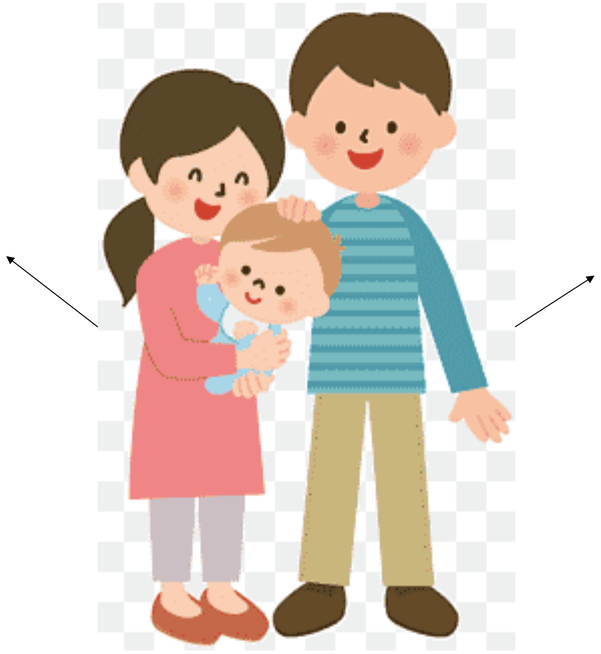
Beta IVS 2.745 het

6 months, male

Hb	12,1
MCV	61,4
MCH	30,7
RDW	10,4
Serum Iron	77
Transferrin	264
Transferrin sat	20%
Ferritin	15
HbA2	5,7
HbF	0,7

Alpha normal

Beta IVS 2.745 het



Hb	16
MCV	87,9
MCH	27,8
RDW	12,5
Serum Iron	72
Transferrin	244
Transferrin sat	20%
Ferritin	175
HbA2	3,1
HbF	0,3

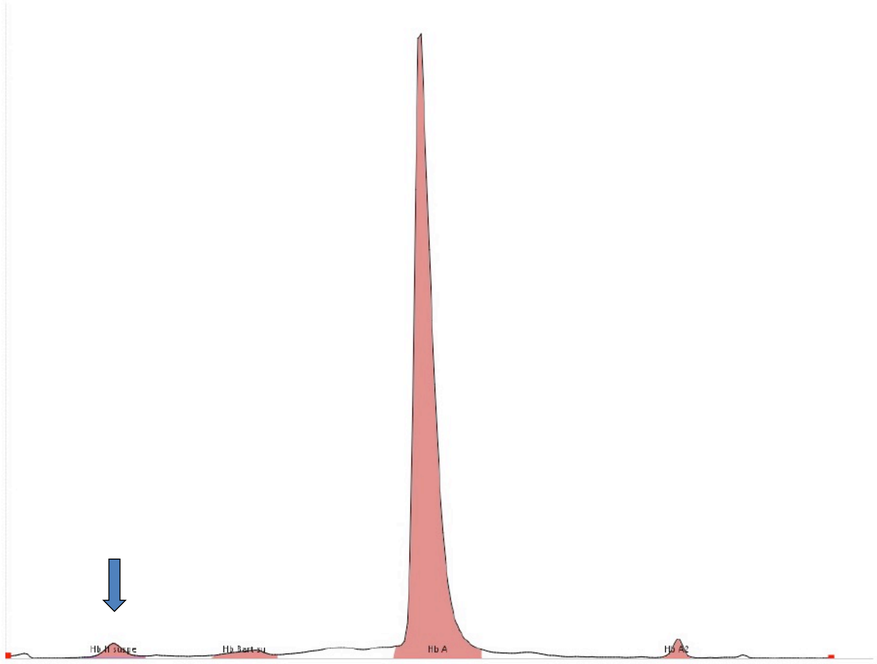
Alpha anti3.7 het

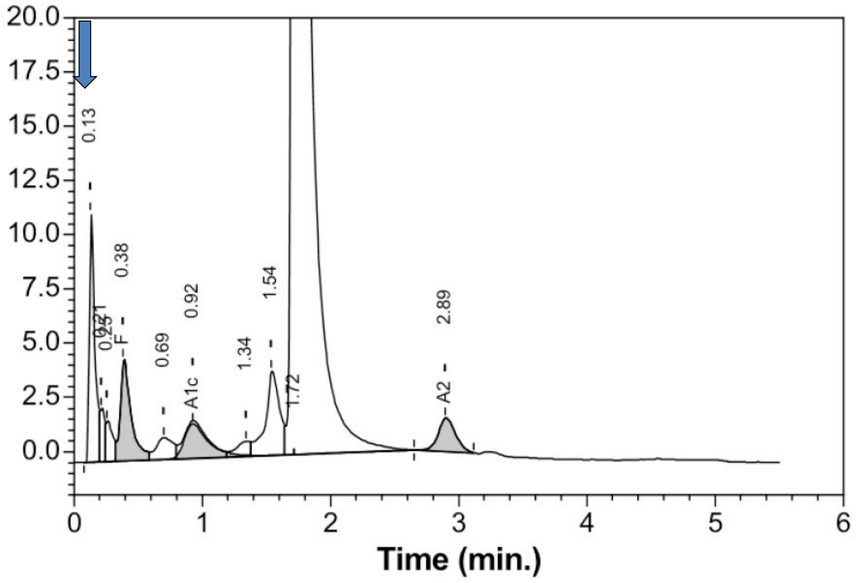
Hb	10
MCV	57,6
MCH	17,4
RDW	11,3

Serum Iron	121
Transferrin	214
Transferrin sat	39%
Ferritin	727

Alpha -3.7/--SEA

HbA2	1,6
HbF	3,1
HbH	3,7





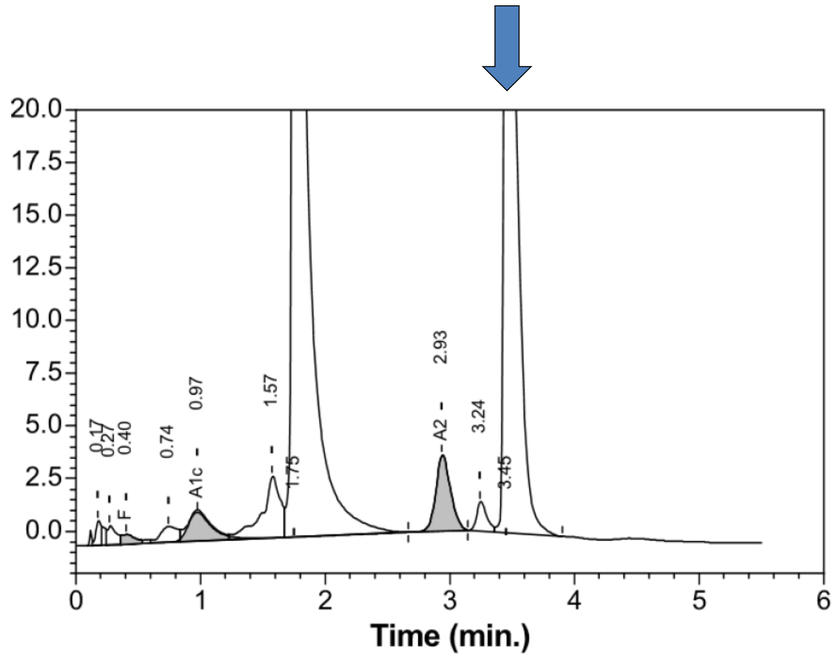
Hb	12,7
MCV	87,5
MCH	28,8
RDW	12,7
Serum Iron	103
Transferrin	241
Transferrin sat	30%
Ferritin	66
HbA2	2,2
HbF	7,6

HPFH

Hb	14,6
MCV	87,5
MCH	28,3
RDW	12,9
Serum Iron	104
Transferrin	275
Transferrin sat	11%
Ferritin	106
HbA2	1,5
HbF	0,5
HbX	1,1

Delta globin variant

Hb	15,5
MCV	83,3
MCH	26,4
RDW	14
Serum Iron	103
Transferrin	181
Transferrin sat	40%
Ferritin	345
HbA2	23,6
HbF	0,1
HbS	33,4



Alpha -3.7 het
HbS het

RIASSUNTO DELLE PIÙ FREQUENTI COMBINAZIONI IN DIAGNOSI PRENATALE

(2) (24)

Genitore portatore di	β0	HbS	HbC/ Hb O-Arab/ HbD-Punjab	HbE	δβ0	HbLepore	β+	α0	α+
β0									
HbS									
HbC/ Hb O-Arab/ HbD-Punjab									
HbE									
δβ0									
Hb Lepore									
β+									
α0									
α+									

In rosso le situazioni con indicazione alla diagnosi prenatale, in giallo le situazioni in cui l'indicazione alla diagnosi prenatale è aperta.

Diagnostica di I e II livello delle Emoglobinopatie

Buone Pratiche SITE