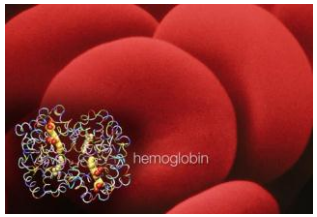
Current Topics in Hemoglobinopathies



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28-29 September 2015
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WHO-TIF MEETING ON THE MANAGEMENT OF HAEMOGLOBIN DISORDERS

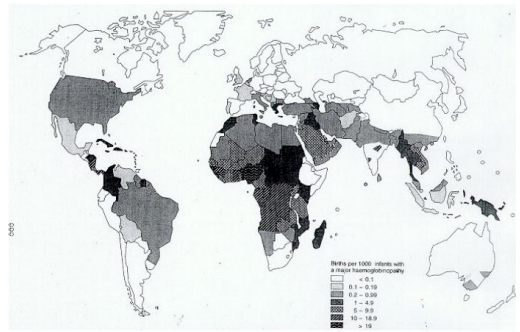


Figure: Global distribution of haemoglobin disorders,

In terms of births of affected infants per 1000 births (WHO, 1996)

World Health Organization

Bulletin of the World Health Organization

Global epidemiology of haemoglobin disorders and derived service indicators

Bernadette Modell, Matthew Darlison

Volume 86, Number 6, June 2008, 480-487

Table 1. Estimated prevalences of carriers of haemoglobin gene variants and affected conceptions

	Demography/2003				% of the populationcarrying			Affected conceptions(per 1000)			Affected births (% of under-5 mortality)
WHO region	Population (millions)	CrudeBirthrate	Annual births (1000s)	Under-5 mortality rate	Significant variant[a]	α[0] thalassaemia[b]	Any variant[c]	Sickle-cell disorders[d]	Thalassaemias[e]	Total	
African	586	39.0	22 895	★168	18.2	41.2	44.4	10.68	0.07	10.74	6.4
American	853	19.5	16 609	27	3.0	4.8	7.5	0.49	0.06	0.54	2.0
Eastern Mediterranean	573	29.3	16 798	★108	4.4	19.0	21.7	0.84	0.70	1.54	1.4
European	879	11.9	10 459	25	1.1	2.3	3.3	0.07	0.13	0.20	0.8
South-east Asian	1 564	24.4	38 139	★83	6.6	44.6	45.5	0.68	0.66	1.34	1.6
Western Pacific	1 761	13.6	23 914	38	3.2	10.3	13.2	0.00	0.76	0.76	2.0
World	6 217	20.7	128 814	★81	5.2	20.7	24.0	2.28	0.46	2.73	3.4

[a] Significant variants include Hb S, Hb C, Hb E, Hb D etc. β thalassaemia, [b] α[0] thalassaemia.[c] α[0] thalassaemia includes heterozygous and homozygous α[0] thalassaemia.[d] Allows for (1) coincidence of α and β variants, and (2) harmless combinations of β variants.[e] Sickle-

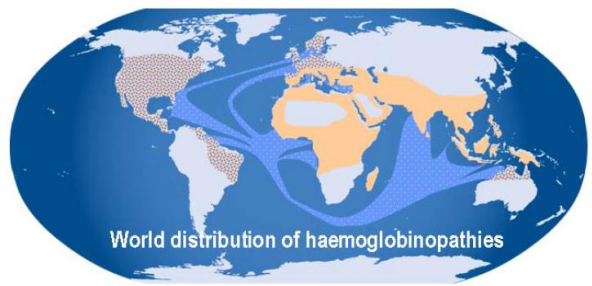


Figure 1 The world distribution of haemoglobinopathies overlaps the geographic distribution of malaria. The prevalence has increased in previously non-endemic areas as a consequence of historical and recent immigration flows, slave-trade, trading activities and colonization. In all these regions there is a high prevalence of a thalassaemia. It is believed that carriers of α thalassaemia are protected against malaria and that natural selection is responsible for elevating and maintaining their gene frequencies.

Harteveld and Higgs *Orphanet Journal of Rare Diseases* 2010, 5:13

How malaria escapes effective immunological responses

P falciparum exports PfEMP1 proteins and concentrate them in knob-like protrusions on the surface of their host RBCs. This enables a large mass of parasitized RBCs to sequester in venous microvessels thereby avoiding splenic clearance from the bloodstream.

Silent alpha-thal carriers show an 18% reduction in PfEMP1 on the surface of the parasitized erythrocyte relative to wild type RBC's. Hemoglobin H disease carriers show a 43% reduction.

Data suggests that α-globin deletions also ameliorate the pro-inflammatory effects of cytoadherence.

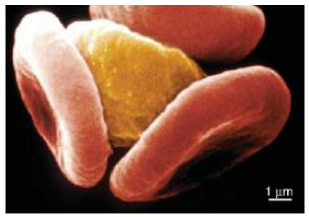


Figure 3. Three-dimensional photograph of normal red cells aggregating around a malaria-infected red

Cold Spring Harb Perspect Med 2012;2:a011692

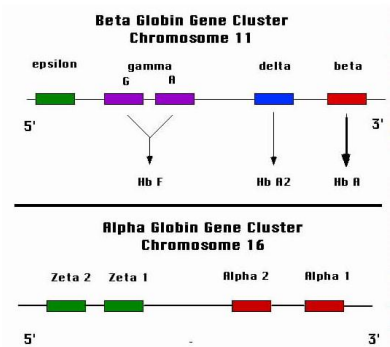
Epidemiology

- Estimations suggest that in excess 300 000 children are born each year with a severe inherited disorder of hemoglobin
- Approximately 80 % occur in low- or middle-income countries
- These pathologies present an uneven distribution in high-frequency populations
- Complex population genetics
- The epidemiological burden is still unknown

Weatherall D.J. Blood.2010. 115: 4431-4336

Hemoglobin (Hgb) Components

- Epsilon and Zeta-globins are found in embryonic Hgb's
- Fetal Hgb (Hb F) comprises two alpha-globin chains and two gamma-globin chains.
- After the 1st year of age the Hgb that accounts for 96% of the hemoglobin of an RBC is typically Hb A--two alpha-globin chains and two beta-globin chains.
- A post-natal Hgb that typically comprises not more than 3.5% of the total RBC hemoglobin is Hb A_2—two alpha chains and two delta-globin chains



Abnormalities of hemoglobin

Encompass all genetic diseases of hemoglobin

✓ **Qualitative abnormalities**: Hemoglobinopathies
- Structural abnormalities, mutation or substitution of one or more amino acids, often leading to a charge modification of the hemoglobin molecule
- Frequent Hb variants: β-globin : **S**, **C**, **D^{Punjab}**, **E**
- Rare variants :α, δ chains

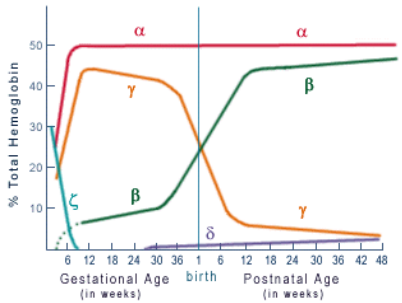
✓ **Quantitative abnormalities**: Thalassemias
- Synthesis or regulation abnormalities :
  - ↓β-globin ⇨ β Thalassemias
  - ↓ α-globin ⇨ α Thalassemias
  - ↓ δβ-globins ⇨ δβ Thalassemias

✓**Combinations**: people can inherit **both** a qualitative and a quantitative abnormality

Clinical symptoms
- **Highly variable clinical manifestations ranging from mild hypochromic anemia to severe anemia with multiorgan involvement.**
- **Lifelong transfusion is used as supportive treatment in cases of sever anemia**

Globin production as a function of gestational age

The differences as to when alpha-globin and beta-globin production becomes prominent explains why a fetus with no working copy of the alpha-globin gene is in trouble by the 2nd trimester of gestation. In contrast, the fetus with no working copy of the beta-globin gene* is in difficulty postnatally once the gamma-globin production decreases.



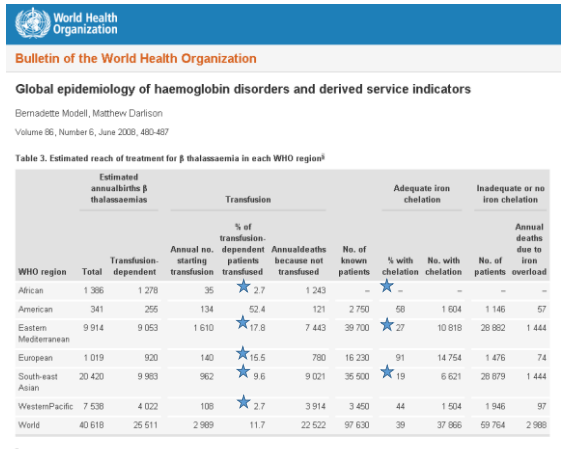


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Table 3. Estimated reach of treatment for β thalassaemia in each WHO region[a]

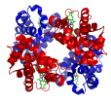
	Estimated annualbirths β thalassaemias		Transfusion				Adequate iron chelation		Inadequate or no iron chelation	
WHO region	Total	Transfusion-dependent	Annual no. starting transfusion	% of transfusion-dependent patients transfused	Annualdeaths because not transfused	No. of known patients	% with chelation	No. with chelation	No. of patients	Annual deaths due to iron overload
African	1 386	1 278	35	★ 2.7	1 243	–	★ –	–	–	–
American	341	255	134	52.4	121	2 750	58	1 604	1 146	57
Eastern Mediterranean	9 914	9 053	1 610	★ 17.8	7 443	39 700	★ 27	10 818	28 882	1 444
European	1 019	920	140	★ 15.5	780	16 230	91	14 754	1 476	74
South-east Asian	20 420	9 983	962	★ 9.6	9 021	35 500	★ 19	6 621	28 879	1 444
WesternPacific	7 538	4 022	108	★ 2.7	3 914	3 450	44	1 504	1 946	97
World	40 618	25 511	2 989	11.7	22 522	97 630	39	37 866	59 764	2 988

- Objective #1 To develop an understanding of the advantages of Capillary Electrophoresis over other technologies in assessing hemoglobin
- Objective #2 To review the effect of alpha globin deletions on HbE and HbA2 levels
- Objective #3 To acquire knowledge regarding globin variants not routinely identified by hospital laboratories

Objective #1

To develop an understanding of the advantages of Capillary Electrophoresis over other technologies in assessing hemoglobin

Methods available for Hemoglobin analysis

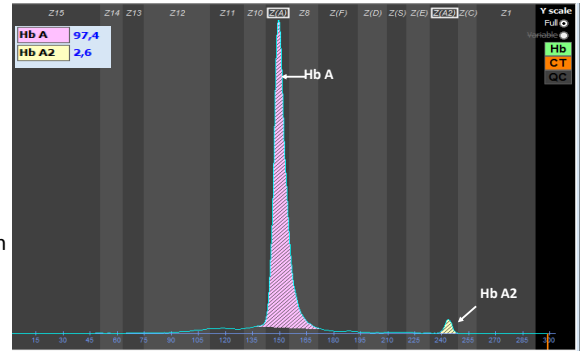


- Gel electrophoresis (alkaline, acid)
- Methods for detection of specific hemoglobins (sickling test for HbS, isopropanol denaturation for unstable hemoglobins)
- IsoElectric Focusing (IEF) on gel (time-consuming; requires experienced interpreter; qualitative)
- HPLC
- Capillary electrophoresis (CE)

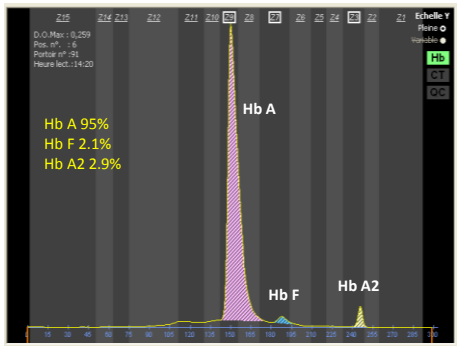
Due to the existence of more than 1000 variants and that some rare variants co-migrate with normal hemoglobins and/or common pathological variants, several methods must be used in combination for diagnosis.

Hemoglobin electrophoresis on CE systems

- HbA, HbA_2 & HbF are detected automatically
- Relative quantitation of the different fractions
- Centered pattern
- Variant separation in a specific zone (Z1 to Z15)



21 yr old pregnant woman: Hgb 13.1 g/dl, Hct 38.6%



Normal pattern, Hb F slightly increased due to the pregnancy

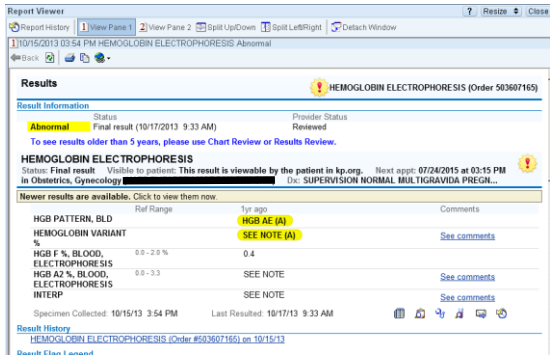
Comparison of HPLC to Capillary Electrophoresis

HPLC

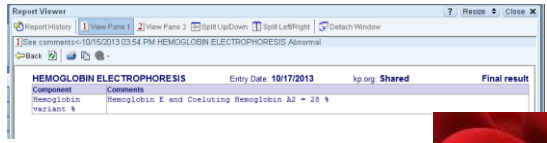
- HPLC cannot separate HbE and HbA2 or E and Hb Lepore
- In presence of HbS, HbA2 is falsely elevated given HbS1C elutes with HbA2. The common delta-globin variant in Blacks HbA2' also elutes with HbS
- ↑HbA1C makes HbF quantification inaccurate
- Detection of low levels of Hb Barts is unreliable and unreproducible using HPLC
- ↑ bilirubin may mascaraed as HbBarts
- Using HPLC, Hb New York cannot be differentiated from HbA. They elute together.

Capillary Electrophoresis

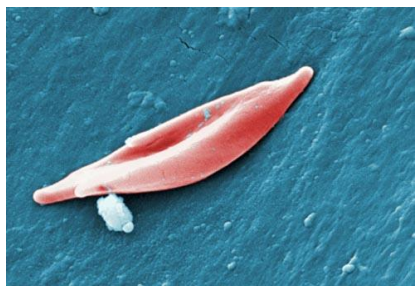
- HbE and Lepore can easily be differentiated from HbA2
- HbA2 is accurate regardless HbS presence or absence
- Despite glycation, HbA1C migrates with HbA and HbS1C migrates with HbS
- Uses higher voltage so run times are shorter
- Smaller sample size thus better for neonatal/pediatric cases
- ~10,000 samples per automated run
- More operator friendly



HPLC cannot separate HbE and HbA2



Sickle cell



Using HPLC, when HbS is present HbA2 is falsely elevated given HbS1C elutes with HbA2

- HbA1C is produced by nonenzymatic addition of a glucose molecule to the N-terminal valine residue of the β-globin chain
- This glycation changes β-globin's structure and decreases the positive charge of HbA
- HbS1C is also produced by adding a glucose molecule to the N-terminal valine residue of the βS-globin chain

Sofronescu, Williams, et al., Clin Chem, 57(2) 153-7, 2011

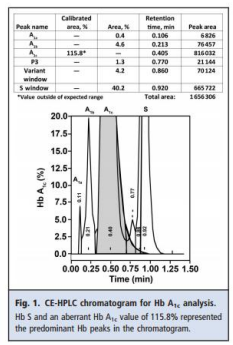
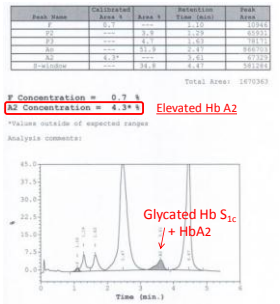
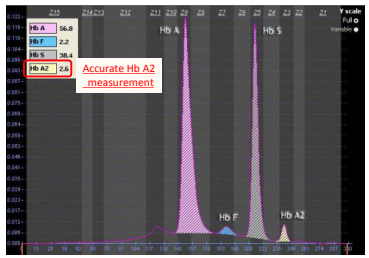


Fig. 1. CE-HPLC chromatogram for Hb A_{1c} analysis.
Hb S and an aberrant Hb A_{1c} value of 115.8% represented the predominant Hb peaks in the chromatogram.

In contrast to HPLC, CE has no interference of glycated Hb S1c on the Hb A2 level



HPLC

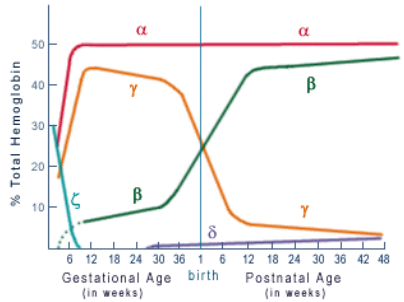


CAPILLARY ELECTROPHORESIS

➢No risk of falsely elevated Hb A2 with CE technologies in case of Hb S presence

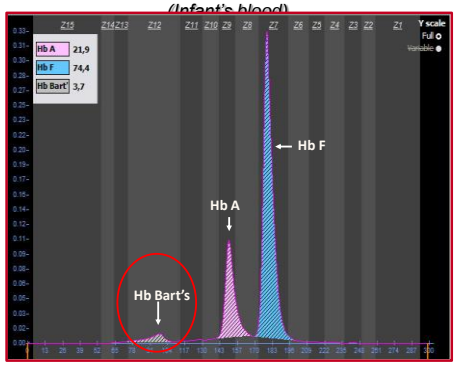
Consequences of alpha-thalassemia

With a relative shortage of α-globin chains, γ-globins form a tetramer named Hb Barts. If the α-globin chains are more significantly scarce the β-globins form a tetramer named Hb H. These homo-tetramers do not deliver oxygen.



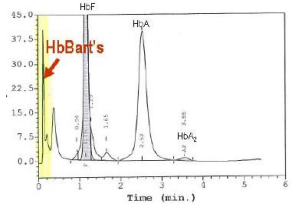
α-thalassemia trait with Hb Bart's

(Infant's blood)



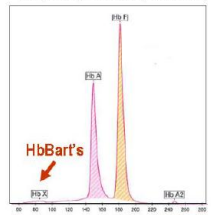
new born --/αα

HPLC



Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.8	0.54	9817
F	63.6*	---	1.15	486314
P3	---	4.1	1.29	30987
P3	---	7.1	1.65	53816
A0	---	59.2	2.52	557115
A2	0.9*	---	3.55	10497

Capillary Electrophoresis



Fractions	%
HbX	0.7
HbA	39.2
HbF	59.6
HbA2	0.5

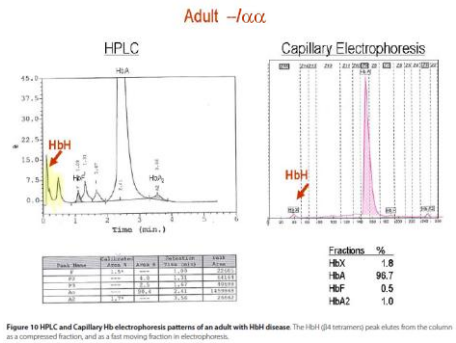


Figure 10 HPLC and Capillary Hb electrophoresis patterns of an adult with HbH disease. The HbH (β4 tetramers) peak elutes from the column as a compressed fraction, and as a fast moving fraction in electrophoresis.

HbH inclusions can be seen when three alpha-globin genes are deleted.

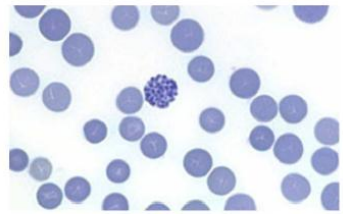


Figure 13 An inclusion body positive cell seen in Brilliant Cresyl Blue stained red cells of a α^0-thalassaemia carrier. Inclusion Bodies are β4-tetramers precipitating on the red cell membrane, which damages the membrane and induces haemolysis. HbH is unstable and inclusion body positive cells are more difficult to find in older blood samples. The number of inclusion body cells seen after staining is much lower in α^0-thalassaemia carriers than in patients with HbH disease (1 in 5-10 fields versus several per field at 1000× microscopic magnification).

Hemoglobin New York

HBB c.341T>A aka Val113Glu

- 2nd most common β-globin variant among those Americans of Chinese ancestry
- Using HPLC, Hb New York elutes with HbA
- Hb New York has been found in combination with β-thalassemia and HbE with no clinical consequence
- Sickle crises, splenic infarct, acute chest syndrome reported in compound heterozygotes for HbS / Hb New York

HPLC Disadvantages

- HPLC cannot separate HbE and HbA2
- In presence of HbS, HbA2 is elevated due to HbS1C
- ↑HbA1C makes HbF quantification inaccurate
- HPLC's detection of low levels of Hb Barts is unreliable and unreproducible.
- ↑ bilirubin may mascaraed as Hb Barts

Is Capillary Electrophoresis a suitable tool for Hemoglobinopathy diagnosis?

Requirements for a good method of Hemoglobinopathy diagnosis:

✓Accurate and precise separation of hemoglobin types, together with reliable quantitation, are essential for differential diagnosis of diseases.

✓Ability to separate an quantitate **Hb A2**, Hb F, Hb H, Hb Barts, HbS, HbC, HbD and HbE which are important parameter requires for diagnosis of thalassemias and hemoglobinopathies

- Capillary electrophoresis separates ions based on their electrophoretic mobility with the use of an applied voltage.
- **The electrophoretic mobility is dependent upon the charge of the molecule, the viscosity, and the atom's radius.**
- If two ions are the same size, the one with greater charge will move the fastest.
- For ions of the same charge, the smaller particle has less friction and overall faster migration rate.
- Capillary electrophoresis is used most because it gives faster results and provides high resolution separation.

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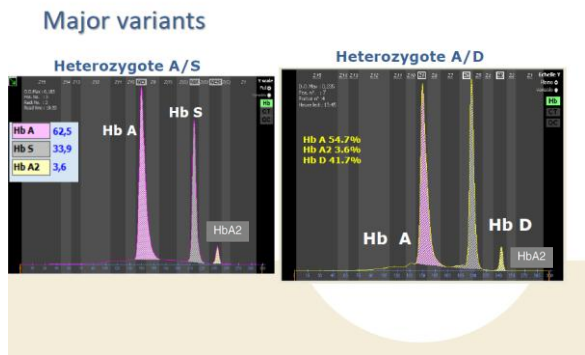
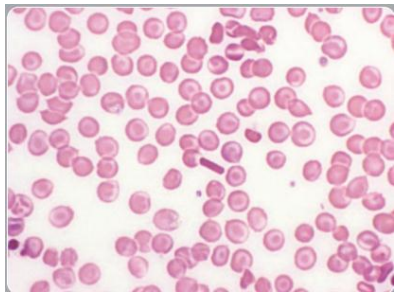
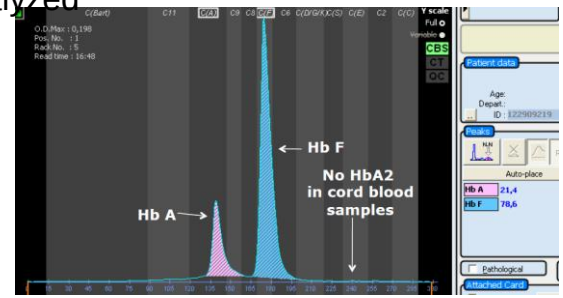


HbC crystal



CE is able to use a small aliquot of blood so that even an umbilical cord sample can be analyzed



β-thalassemia trait

Clinical context

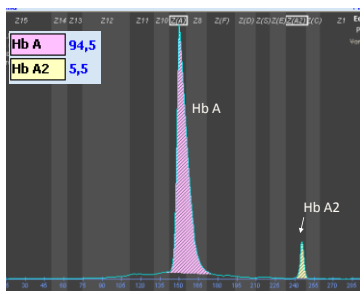
11 yr old Palestinan child

Hematological parameters

- WBC	7.71	G/L	
- RBC	5.18	T/L	
- Hb	9.5	g/dL	(11.5 - 15.5)
- Hct	29.6	%	(35 - 45)
- MCV	57.1	fL	(77 - 95)

Iron status

- Iron	15 µmol/L	
- Transferrin	2.13	g/L
- Ferritin	95 µg/L	



Elevated HbA2, anemia and microcytosis indicate a beta-thalassemia

Advantages of CE

1. Allows clear separation of HbC, HbE, and Lepore from A2
2. Migration difference between HbS and HbD enabling more accurate measurements
3. A perfect focalization of HbF between HbA and HbS allowing precise quantification of F
4. A very good focalization of HbA2 allowing automatic identification and quantification
5. Excellent separation of A2 variants
6. CE has the fewest number of variants that can be confused with the most common variants HbS, HbC, and HbE [American Society of Pathology, 2011]

Hemoglobinopaty Program

Printed On: 9/3/2015 10:23:00 AM
Page: 1

Prenatal Screening Case Summary Report

Number of Case(s): 1

Home Facility: OAK

Downloaded On: 8/27/2015

HC Ethnicity: Filipino/Philippine

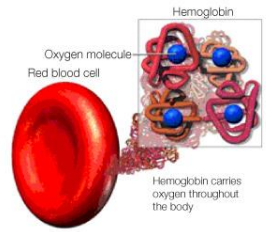
HC Race: Asian

Ferritin: ☐

Order Date	FAC	E4Date	TestCode	Test Name	Reading	Res Unit	Out Range	Text
8/21/2015	OAK	8/22/2015	2000020	WBC	9.0	K/uL		
8/21/2015	OAK	8/22/2015	2000040	RBC	4.94	M/uL		
8/21/2015	OAK	8/22/2015	2000060	HEMOGLOBIN	11.5	g/dL		
8/21/2015	OAK	8/22/2015	2000080	HEMATOCRIT	35.6	%		
8/21/2015	OAK	8/22/2015	2000100	MCV	72	fL	L	
8/21/2015	OAK	8/22/2015	2000150	RDW	15.7	%		
8/21/2015	OAK	8/22/2015	2000160	PLATELET C	321	K/uL		
8/22/2015	OAK	8/22/2015	1000794	HGBPROF	HGB AA			
8/22/2015	OAK	8/22/2015	1000820	FERRITIN	224	ng/mL		
8/22/2015	OAK	8/22/2015	3001135	HGB F	1.2	%		
8/22/2015	OAK	8/22/2015	3001136	HGBNPENTER	SEE			Pattern consistent with Alpha Thalassemia trait.
8/22/2015	OAK	8/22/2015	3001150	HGB A2	5.3	%	H	
8/22/2015	OAK	8/22/2015	1480000	HGB A	93.5	%		

Information required for interpretation

- **Correct interpretation of a result requires both hemoglobin electrophoresis profile and other blood indices & information**:
  - Hgb, Hct, mean red cell volume (MCV), mean corpuscular hemoglobin (MCH) etc. to assess anemia, microcytosis, and/or hypochromia
  - Iron status (in particular for the decrease of Hb A2 and MCV in iron deficiency)
  - Patient's age, geographical /ethnic origin and family history
  - Transfusion history of the patient



Hemoglobinopaty Program

Printed On: 9/3/2015 10:23:00 AM
Page: 1

Prenatal Screening Case Summary Report

Number of Case(s): 1

Home Facility: OAK

Downloaded On: 8/27/2015

HC Ethnicity: Non Hispanic/Non Latino

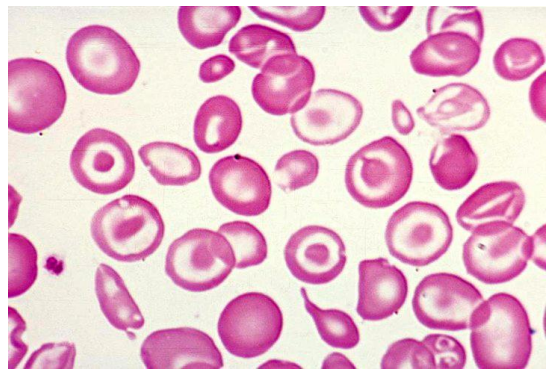
HC Race: Black/African American

Ferritin: ☐

8/21/2015	OAK	8/21/2015	3001135	HGB F	1.8	%		
8/21/2015	OAK	8/21/2015	3001136	HGBNPENTER	SEE			If no transfusion, pattern consistent with Hemoglobin S Trait with Iron Deficiency and/or an underlying Alpha Thalassemia gene deletion.
8/21/2015	OAK	8/21/2015	3001150	HGB A2	3.4	%	H	
8/21/2015	OAK	8/21/2015	1480000	HGB A	60.6	%		
8/21/2015	OAK	8/21/2015	1480001	HGB S	34.2	%	H	
8/21/2015	OAK	8/21/2015	2000020	WBC	7.0	K/uL		
8/21/2015	OAK	8/21/2015	2000040	RBC	4.21	M/uL		
8/21/2015	OAK	8/21/2015	2000060	HEMOGLOBIN	11.5	g/dL		
8/21/2015	OAK	8/21/2015	2000080	HEMATOCRIT	33.0	%	L	
8/21/2015	OAK	8/21/2015	2000100	MCV	78	fL	L	
8/21/2015	OAK	8/21/2015	2000150	RDW	13.4	%		
8/21/2015	OAK	8/21/2015	2000160	PLATELET C	253	K/uL		
8/23/2015	OAK	8/21/2015	2000020	WBC	8.9	K/uL		
8/23/2015	OAK	8/21/2015	2000040	RBC	4.29	M/uL		
8/23/2015	OAK	8/21/2015	2000060	HEMOGLOBIN	11.7	g/dL		
8/23/2015	OAK	8/21/2015	2000080	HEMATOCRIT	33.1	%	L	
8/23/2015	OAK	8/21/2015	2000100	MCV	77	fL	L	
8/23/2015	OAK	8/21/2015	2000150	RDW	13.3	%		

Objective #2

To review the effect of α-globin deletions on HbE and HbA2 levels



Alpha-globin Deletions account for the majority of Alpha Thalassemias

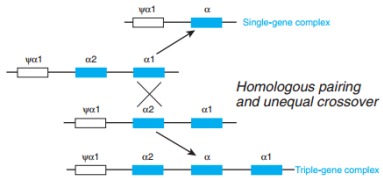


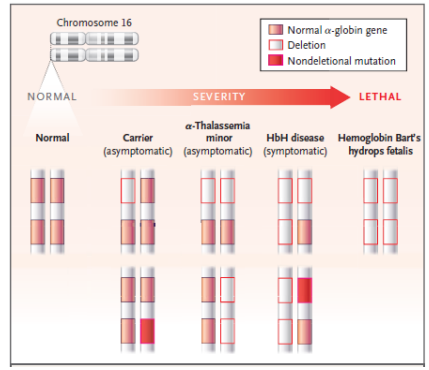
Figure 11-8 ■ The probable mechanism underlying the most common form of α-thalassemia, which is due to deletions of one of the two α-globin genes on a chromosome. Misalignment, homologous pairing, and recombination between the α1 gene on one chromosome and the α2 gene on the homologous chromosome result in the deletion of one α gene. (Redrawn from Orkin SH: Disorders of hemoglobin synthesis: the thalassemias. In Stamatoyannopoulos G, Nienhuis AW, Leder P, Majerus PW [eds]: The Molecular Basis of Blood Diseases. Philadelphia, WB Saunders, 1987, pp 106-126.)

Non-Allelic Homologous Recombination

Thompson & Thompson Genetics in Medicine, 2007

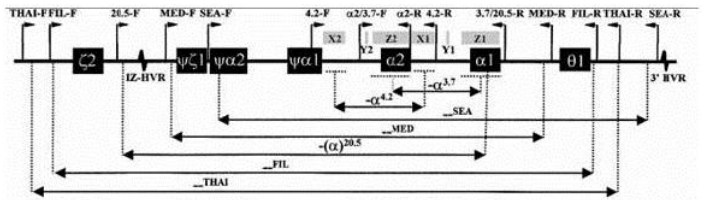
Alpha-Thalassemias

The number of working copies of alpha-globin genes that are present in a given person is typically four. But there are individuals with more than four and those with less. Whenever less, this is a type of alpha-thalassemia.



NEJM 371: 1909, 2014

Common Alpha-globin Deletions

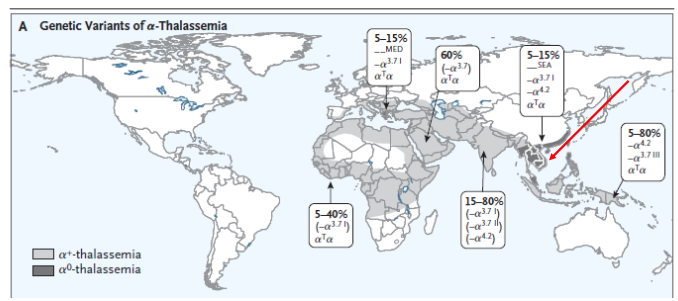


Schematic representation of the α-globin gene cluster, indicating extents of the 7 deletions and relative positions of the primers (except for the control LIS1-F and LIS1-R primers, which are located on a different chromosome). Locations of X, Y, and Z sequence homology boxes and hypervariable regions (HVRs) are also shown.

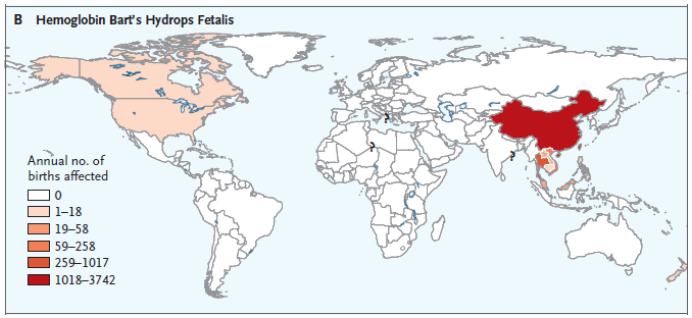
Cerner | Clinical Bioinformatics Ontology www.cerner.com

Review α-globin deletion locations

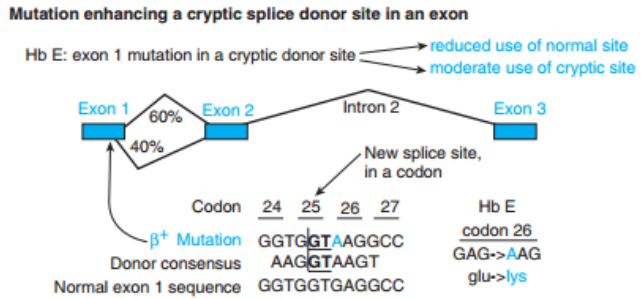
The NEW ENGLAND JOURNAL of MEDICINE



α-Thalassemia major Hydrops Fetalis



HbE is a qualitative beta-globin variant that is also thalassemic



Thompson & Thompson Genetics in Medicine, 2007

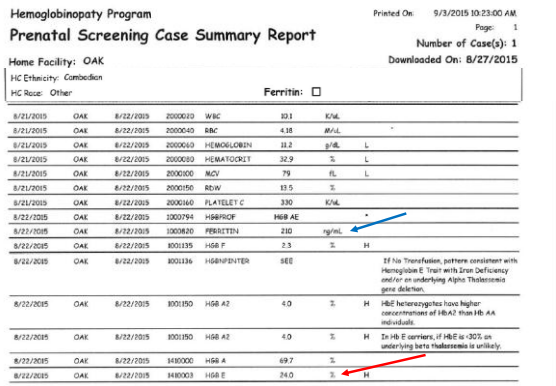
E trait: The % HbE lowers as the number of α-globin genes present goes down

α-Genotype	HbE%#	MCV#	n	Notes
αα/αα	29.2%	78.2	80	
-α/αα	27.5%	81.2	51	Silent carriers without HbE typically have MCV between 77 and 82
--/αα	21.4%	69.5	21	
-α /-α	20.1%	73.9	9	
--/-α	14.2%	54	18	

Medians

HbA_2% is typically ≤ 4.3%

Ann Hematol (2003) 82: 612-616



The effect of α-globin deletions on HbA2 %:

α-globin deletions lower the HbA2%. The % A2 lowers as the number of α-globin genes present lowers

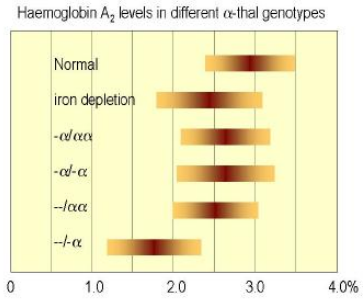


Figure 12 Mean and standard deviation of HbA_2 in different α-thalassaemia genotypes.

Harteveld and Higgs *Orphanet Journal of Rare Diseases* 2010, 5:13

HbA2: The % HbA2 lowers slightly as the number of α-globin genes present goes down

α-Genotype	HbA_2%#	n	Ferritin	Notes
αα/αα	2.57 ± 0.23%*	107 ♂	≥ 30 µg/L	
$-\alpha^{3.7}/-\alpha^{3.7}$	2.44 ± 0.20%*	65 ♂	≥ 30 µg/L	
	2.48 ± 0.25%@	307♀	≥ 30 µg/L	^
	2.38 ± 0.25%	123♀	≥ 15 but <30 µg/L	^
	2.31 ± 0.23%@	75 ♀	< 15 µg/L	

Means ± SD
* P < 0.0001
@ P < 0.0001
^ P = 0.01

ISRN Hematology, 2013, ID 858294 http://dx.doi.org/10.1155/2013/858294

Hb Constant Spring, -α3.7del and δβ-thal trait in a 33 y.o. male of Chinese ancestry

Malaysian J Pathol 2012 34(1): 57-62

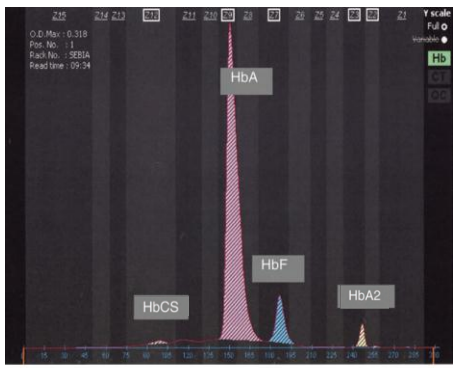


FIG. 1: Capillary electrophoresis analysis showing presence of Hb CS peak.

Objective #3

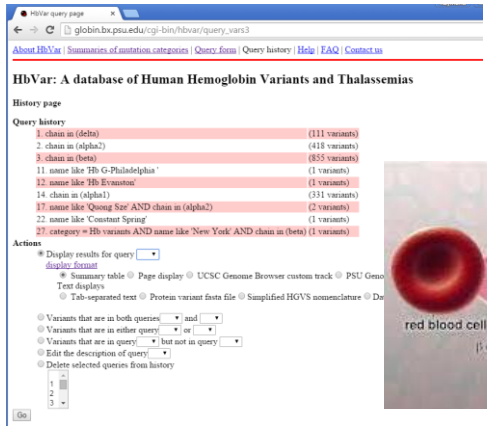
To acquire knowledge regarding globin variants not routinely identified by hospital laboratories.

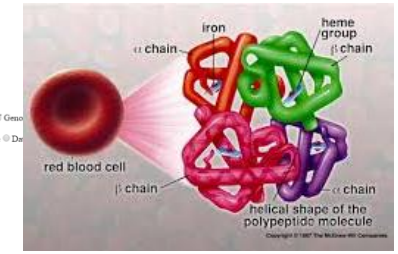
Why are globin variants complicated?

- Sheer number
  http://globin.bx.psu.edu/cgi-bin/hbvar/query_vars3

  HbVar: A database of Human Hemoglobin Variants and Thalassemias

  tallies:
  - **418 α2 variants**
  - **331 α1 variants**
  - **855 β variants**
  - **111 δ variants**
- Patient ethnicity, globin variant stability, and status of the remaining loci need to be considered when evaluating presence of a variant
- Also important in interpretation is the % variant out of the total hemoglobin
- While most do not, several variants have thalassemic properties: Hb E, Hb Lepore, Hb Constant Spring, and Hb Quong Sze





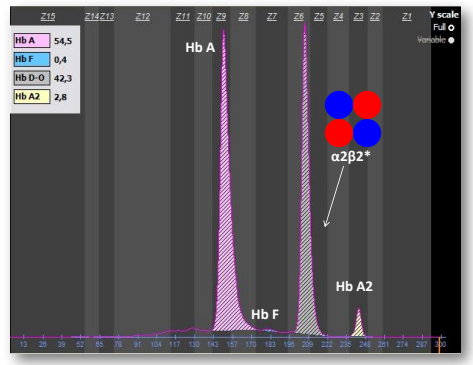
Number of variants…

http://globin.bx.psu.edu/cgi-bin/hbvar/query_vars3

HbVar: A database of Human Hemoglobin Variants & Thalassemias:

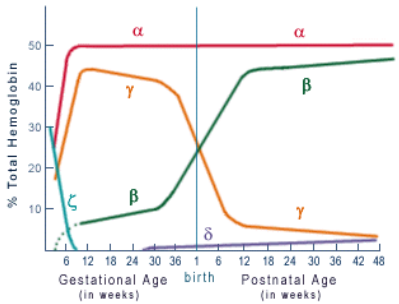
111 δ variants

variant of the β chain



Globin production as a function of gestational age

In general, delta globin variants are clinically insignificant. Yet the total Hb A2 is a vital piece of information for assessing beta-thalassemia status, so presence of a delta globin variant need to be added to the traditional delta globin's A2 to get the total Hb A2.



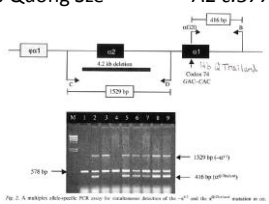
Although CE has the fewest number of variants that can be confused with the common variants… it is not a panacea

Order Date	FAC	E4Date	TestCode	Test Name	Reading	Res Unit	Out Range	Text
9/4/2015	ELG	9/4/2015	1000794	HGBPROF	HGB AD		*	
9/4/2015	ELG	9/4/2015	1000820	FERRITIN	8	ng/mL	L	
9/4/2015	ELG	9/4/2015	1001135	HGB F	<1.0	%		
9/4/2015	ELG	9/4/2015	1001136	HGBNPINTER	SEE			If no transfusion, pattern consistent with Hemoglobin D Trait.
9/4/2015	ELG	9/4/2015	1001150	HGB A2	3.2	%		
9/4/2015	ELG	9/4/2015	1410000	HGB A	57.1	%		
9/4/2015	ELG	9/4/2015	1410004	HGB D	39.7	%	H	
9/4/2015	ELG	9/4/2015	2000020	WBC	9.5	K/uL		
9/4/2015	ELG	9/4/2015	2000040	RBC	4.91	M/uL		
9/4/2015	ELG	9/4/2015	2000060	HEMOGLOBIN	11.7	g/dL		
9/4/2015	ELG	9/4/2015	2000080	HEMATOCRIT	35.2	%		
9/4/2015	ELG	9/4/2015	2000100	MCV	72	fL	L	
9/4/2015	ELG	9/4/2015	2000150	RDW	15.4	%		
9/4/2015	ELG	9/4/2015	2000160	PLATELET C	323	K/uL		

Most Common Globin Variants in Guangdong

Alpha Variants

- (1) Hb Constant Spring *A2 c.427T>C*
- (2) Hb Q Thailand *A1 c.223G>C*
- (3) Hb G Honolulu *A2 c.91G>C*
- Hb Quong Sze *A2 c.377T>C*



Beta Variants

- (1) Hb E *HBB c.79G>A*
- (2) Hb New York *HBB c.341T>A*
- (3) Hb J-Kaohsiung *HBB c.179A>C*

Laotian Beta Variants

- (1) Hb E *HBB c.79G>A*
- (2) Hb Minneapolis-Laos *HBB c.356T>A*
- (3) Hb Hope *HBB c.410G>A*
- (4) Hb Phou Bia *HBB c.295G>C*

Lou, Wang, et al. Hemoglobin, (38)4: 282, 2014

Sanchaisuriya, et al. Euro J Haematol, (74)3: 221. 2005

Hb Quong Sze Globin Variant in Chinese

a highly unstable α-globin chain

Table 1. α-Globin genotypes in 319 patients with Hb H disease from California, Hong Kong, and Ontario[31,33,41]

Nondeletional	53	17
(−−SEA/$\alpha\alpha^{Constant\ Spring}$ [Codon 142 TAA>CAA or Ter→Gln]$_\alpha$)	31	10
(−−SEA/$\alpha\alpha^{Quong\ Sze}$ [Codon 125 CTG>CCG or Leu→Pro]$_\alpha$)	7	2
(−−SEA/$\alpha\alpha^{Codon\ 30\ deletion\ of\ GAG}$$_\alpha$)	3	< 1
(−−TOT/$\alpha\alpha^{Codon\ 30\ deletion\ of\ GAG}$$_\alpha$)	1	< 1
(−−SEA/$\alpha\alpha^{Initiation\ codon\ ATG>AG}$$_\alpha$)	1	< 1
(−−THAI/$\alpha\alpha^{Initiation\ codon\ ATG>AG}$$_\alpha$)	1	< 1
(−−SEA/$\alpha\alpha^{Codon\ 31\ AGG>AAG\ or\ Arg→Lys}$$_\alpha$)	2	< 1
(−−FIL/$\alpha\alpha^{Codon\ 35\ TCC>CCC\ or\ Ser→Pro}$$_\alpha$)	1	< 1
(−−MED/$\alpha\alpha^{IVS\ I\ deletion\ of\ TGAGG}$$_\alpha$)	1	< 1
(−−SEA/$\alpha\alpha^{Codon\ 59\ GGC>GAC\ or\ Gly→Asp}$$_\alpha$)	1	< 1
(−−SEA/$\alpha\alpha^{Pakse\ [Codon\ 142\ TAA>TAT\ or\ Ter→Tyr]}$$_\alpha$)	2	< 1
(−−THAI/$\alpha\alpha^{Constant\ Spring\ [Codon\ 142\ TAA>CAA\ or\ Ter→Gln]}$$_\alpha$)	1	< 1
($\alpha^{Hb\ Sallanches\ [Codon\ 104\ TGC>TAC\ or\ Cys→Tyr]}$ $\alpha\alpha$/$\alpha\alpha^{Hb\ Sallanches}$$_\alpha$)	1	< 1

Chui, et al. Blood (101)3: 791,

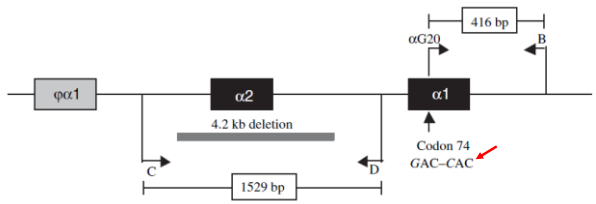
Hb Q Thailand

Table 1. α-Globin genotypes in 319 patients with Hb H disease from California, Hong Kong, and Ontario[31,33,41]

Hb H disease	No. of patients	%
Deletional	266	83
(--^{SEA}/-α$^{3.7}$)	175	55
(--^{SEA}/-α$^{4.2}$)	37	12
(--^{FIL}/-α$^{3.7}$)	36	11
(--^{MED}/-α$^{3.7}$)	8	2
(--^{THAI}/-α$^{3.7}$)	2	< 1
(--^{BRIT}/-α$^{3.7}$)	1	< 1
(--^{SA}/-α$^{3.7}$)	1	< 1
(--$^{de\ novo}$/-α$^{3.7}$)	1	< 1
(--^{SEA}/-α$^{4.2\ Q\text{-}Thailand\ [Codon\ 74\ GAC>CAC\ or\ Asp\rightarrow His]}$)	4	1
(--^{FIL}/-α$^{4.2\ Q\text{-}Thailand\ [Codon\ 74\ GAC>CAC\ or\ Asp\rightarrow His]}$)	1	< 1

Chui, et al. Blood (101)3: 791, 2003

Hb Q Thailand



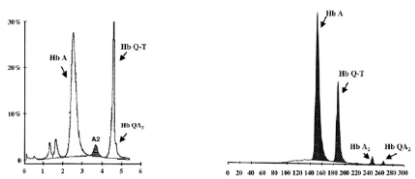
Sanchaisuriya, et al. Euro J Haematol (74): 221, 2005

Hb Q Thailand

Table 2
α-Globin gene haplotypes associated with α$^{Q\text{-}Thailand}$, α^{+}-thalassemia (4.2 kb deletion) and normal α-globin genes in Thai population. Numbers indicate number of alleles associated with each specific haplotype. + and – indicate the presence and absence of each polymorphic site where as 0 indicates deletion. nd = not done.

Globin alleles	Xba I	Bgl I	SM/L	Ace I	Rsa I	α Pst I	θ Pst I	Number of allele
αα*	+	–	S	+	+	–	nd	10
	+	–	S	+	–	–	nd	21
	+	–	S	+	–	+	nd	1
	+	–	S	–	–	–	nd	1
–α$^{4.2}$	+	–	S	+	0	–	–	4
–α$^{Q\text{-}Thailand}$	+	–	S	+	0	–	–	14

* Data from our previous report [15].



Singsanan, et al. BCMB (45): 210, 2010

Most Common Globin Variants in Blacks

Alpha Variants

- Hb G-Philadelphia *A2 c.207C>G* often in cis with the –α 3.7 (*A1*) deletion *A2 Asn68Lys*
- Hb Evanston *A2 c.43T>A* *more* often in cis with the –α 3.7 (*A1*) deletion *A2 Trp14Arg*

Delta Variants

- Hb A2' (HbB2) *HBD c.49G>C Gly16Arg*

Beta Variants

- Hb S
- Hb C

Worth mentioning:

- Hb C Harlem /Hb C Georgetown

The % of the Variant also assists in interpreting which globin chain is involved

- Delta globin variants comprise about ½ of the total HbA2

HEMOGLOBIN ELECTROPHORESIS
Status: Final result Visible to patient: kp.org Next appt: 07/16/2015 at 04:00 PM in Obstetrics, Gynecology [redacted]
[redacted] Dx: PREGNANCY COUNSELING

	Ref Range	6d ago	Comments
HGB PATTERN, BLD		SEE NOTE (A)	See comments
HGB F %, BLOOD, ELECTROPHORESIS	0.0 - 2.0 %	<1.0	
HGB A2 %, BLOOD, ELECTROPHORESIS	0.0 - 3.3 %	1.1	
HEMOGLOBIN ELECTROPHORESIS, INTERPRETATION		SEE NOTE	See comments
HGB A %, BLOOD, ELECTROPHORESIS	%	97.8	
HGB A2 VARIANT %, BLOOD, ELECTROPHORESIS	<=0.0 %	1.1 (H)	

The % of the Variant suggests which globin chain is involved

- Beta globin variants typically comprise between 30-50% of what would otherwise be HbA
- It can be less than 30% if it is unstable or if it has decreased transcription efficiency

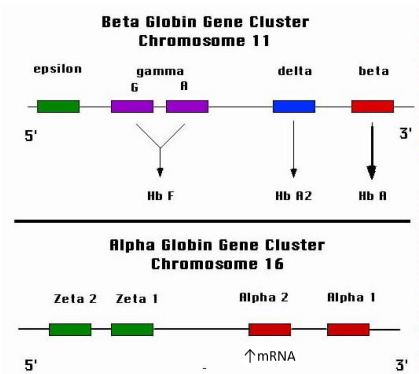
HEMOGLOBIN ELECTROPHORESIS
Status: Final result Visible to patient: kp.org Next appt: 07/15/2015 at 11:15 AM in Obstetrics, Gynecology [redacted]
[redacted] Dx: COUNSELING

	Ref Range	2wk ago	Comments
HGB PATTERN, BLD		HGB AE (A)	
HGB F %, BLOOD, ELECTROPHORESIS	0.0 - 2.0 %	2.1 (H)	
HGB A2 %, BLOOD, ELECTROPHORESIS	0.0 - 3.3 %	4.1 (H)	See comments
HEMOGLOBIN ELECTROPHORESIS, INTERPRETATION		SEE NOTE	See comments
HGB A %, BLOOD, ELECTROPHORESIS	%	68.4	
HGB E %, BLOOD, ELECTROPHORESIS	<=0.0 %	25.4 (H)	

Result Narrative
Hgb Screen reflexed due to MCV < 80.

α-globin Variants

- Each upstream Alpha2 gene produces about twice the amount of mRNA than does Alpha1. Alpha 2 thus codes for about 30% of the total alpha globin made inside the red blood cell.
- That leaves about 20% being made by each Alpha1 gene.



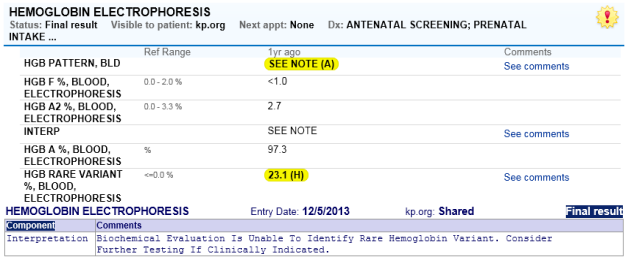
The % of a Variant is not always helpful

HEMOGLOBIN ELECTROPHORESIS
Status: Final result Visible to patient: kp.org Next appt: 07/17/2015 at 03:15 PM in Obstetrics, Gynecology [redacted]
[redacted] Dx: SUPERVISION NORMAL MULTIGRAVIDA PREGN...

	Ref Range	1mo ago	Comments
HGB PATTERN, BLD			
HGB F %, BLOOD, ELECTROPHORESIS	0.0 - 2.0 %	<1.0	
HGB A2 %, BLOOD, ELECTROPHORESIS	0.0 - 3.3 %	3.5 (H)	See comments
HEMOGLOBIN ELECTROPHORESIS, INTERPRETATION		SEE NOTE	See comments
HGB A %, BLOOD, ELECTROPHORESIS	%	66.0	
HGB [redacted]%, BLOOD, ELECTROPHORESIS	<=0.0 %	30.5 (H)	

The % of the α-globin Variant

- The % of an alpha globin variant is somewhat variable:



HEMOGLOBIN ELECTROPHORESIS
Status: Final result Visible to patient: kp.org Next appt: None Dx: ANTENATAL SCREENING; PRENATAL INTAKE ...

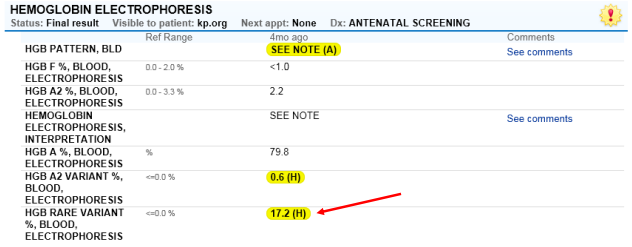
	Ref Range	1yr ago	Comments
HGB PATTERN, BLD		SEE NOTE (A)	See comments
HGB F %, BLOOD, ELECTROPHORESIS	0.0 - 2.0 %	<1.0	
HGB A2 %, BLOOD, ELECTROPHORESIS	0.0 - 3.3 %	2.7	
INTERP		SEE NOTE	See comments
HGB A %, BLOOD, ELECTROPHORESIS	%	97.3	
HGB RARE VARIANT %, BLOOD, ELECTROPHORESIS	<=0.0 %	23.1 (H)	See comments

HEMOGLOBIN ELECTROPHORESIS Entry Date: 12/5/2013 kp.org: Shared Final result

Component	Comments
Interpretation	Biochemical Evaluation Is Unable To Identify Rare Hemoglobin Variant. Consider Further Testing If Clinically Indicated.

The % of the α-globin Variant

- The % of an alpha globin variant is somewhat variable:



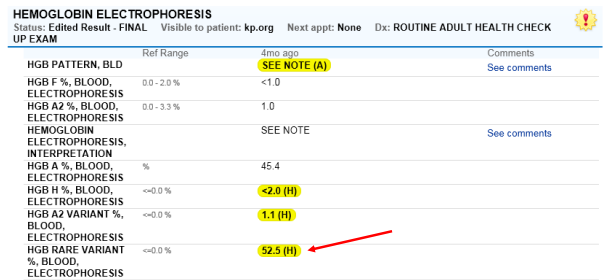
HEMOGLOBIN ELECTROPHORESIS
Status: Final result Visible to patient: kp.org Next appt: None Dx: ANTENATAL SCREENING

	Ref Range	4mo ago	Comments
HGB PATTERN, BLD		SEE NOTE (A)	See comments
HGB F %, BLOOD, ELECTROPHORESIS	0.0 - 2.0 %	<1.0	
HGB A2 %, BLOOD, ELECTROPHORESIS	0.0 - 3.3 %	2.2	
HEMOGLOBIN ELECTROPHORESIS, INTERPRETATION		SEE NOTE	See comments
HGB A %, BLOOD, ELECTROPHORESIS	%	79.8	
HGB A2 VARIANT %, BLOOD, ELECTROPHORESIS	<=0.0 %	0.6 (H)	
HGB RARE VARIANT %, BLOOD, ELECTROPHORESIS	<=0.0 %	17.2 (H)	

Variant interpretation variables:

- Sheer number of identified variants
- Which globin has the nucleotide change?
- If an α-globin variant, is it α2 or α1?
- What is the affinity of the variant globin to its paired globin? e.g. Is the β-variant more positively or negatively charged than normal β-globin?
- What is its stability?
- What is its rate of synthesis?

Practice Case: Which globin Variant?



HEMOGLOBIN ELECTROPHORESIS
Status: Edited Result - FINAL Visible to patient: kp.org Next appt: None Dx: ROUTINE ADULT HEALTH CHECK UP EXAM

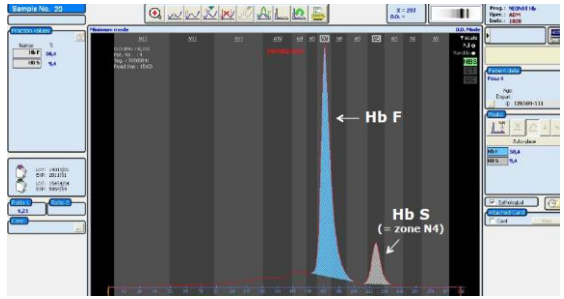
	Ref Range	4mo ago	Comments
HGB PATTERN, BLD		SEE NOTE (A)	See comments
HGB F %, BLOOD, ELECTROPHORESIS	0.0 - 2.0 %	<1.0	
HGB A2 %, BLOOD, ELECTROPHORESIS	0.0 - 3.3 %	1.0	
HEMOGLOBIN ELECTROPHORESIS, INTERPRETATION		SEE NOTE	See comments
HGB A %, BLOOD, ELECTROPHORESIS	%	45.4	
HGB H %, BLOOD, ELECTROPHORESIS	<=0.0 %	<2.0 (H)	
HGB A2 VARIANT %, BLOOD, ELECTROPHORESIS	<=0.0 %	1.1 (H)	
HGB RARE VARIANT %, BLOOD, ELECTROPHORESIS	<=0.0 %	52.5 (H)	

Practice Case: Which globin Variant?

HEMOGLOBIN ELECTROPHORESIS
Status: Final result Visible to patient: kp.org Next appt: 07/09/2015 at 10:10 AM in Obstetrics, Gynecology [redacted]
[redacted] Dx: SUPERVISION NORMAL MULTIGRAVIDA PREGN...

	Ref Range	6mo ago	Comments
HGB PATTERN, BLD		SEE NOTE (A)	See comments
HGB F %, BLOOD, ELECTROPHORESIS	0.0 - 2.0 %	<1.0	
HGB A2 %, BLOOD, ELECTROPHORESIS	0.0 - 3.3	SEE NOTE	See comments
HGB A %, BLOOD, ELECTROPHORESIS	%	97.7	
HGB A2 VARIANT %, BLOOD, ELECTROPHORESIS	<=0.0 %	2.3 (H)	

Practice case: This CE result is on a full-term gestation neonate. What is the suspected dx?



Practice Case: HbA = 72.7% Is the unidentified variant an α-, β-, or δ-globin variant?

HEMOGLOBIN ELECTROPHORESIS
Status: Final result Visible to patient: kp.org Next appt: None [redacted]
Newer results are available. Click to view them now.

	Ref Range	3yr ago	Comments
HGB PATTERN, BLD		SEE NOTE (A)	See comments
HEMOGLOBIN VARIANT %		SEE NOTE (A)	See comments
HGB F %, BLOOD, ELECTROPHORESIS	0.0 - 2.0 %	0.6	
HGB A2 %, BLOOD, ELECTROPHORESIS	0.0 - 3.3 %	2.7	
INTERP		******	

Component	Comments
Hemoglobin variant %	Unidentified Hemoglobin = 24 %