Thalassaemia/Haemoglobinopathy Antenatal screening

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SA PATHOLOGY

Types of screening

1. Premarital screening
2. Preconception screening

- Indicated for at risk groups
- Pre-implantation genetic testing

3. Antenatal screening
4. Prenatal screening
5. Newborn screening

SA PATHOLOGY

Prenatal diagnosis

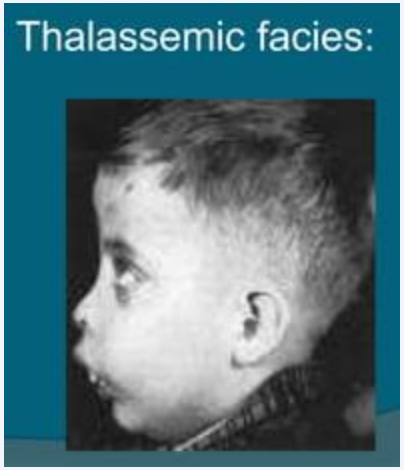
- Performed only when couple identified as having 1:4 or higher risk of a clinically significant disorder in the child.
- Genetic testing is expensive and the genetics can be complicated.
- PND for thalassaemia/haemoglobinopathy is best performed on CVS (i.e. first trimester). Amniocentesis requires culture to get sufficient DNA for analysis– 2 week delay.
- Timing – important. Need 2-4 weeks for molecular work-up.

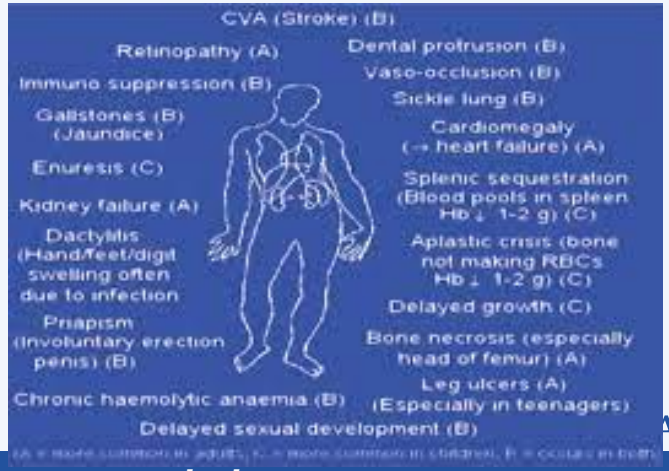
Why screen?

Screen for significant thalasaemia syndromes and structural variants- most common and important- HbS

1. To detect and prevent diseases that are life threatening and causes morbidity, low quality of life and shorten life expectancy
2. Maternal morbidity
3. Options- genetic counselling, prenatal diagnosis, preimplantation genetic testing

Hb Barts- $Hb\gamma_4$





A PATHOLOGY

Who do you screen?

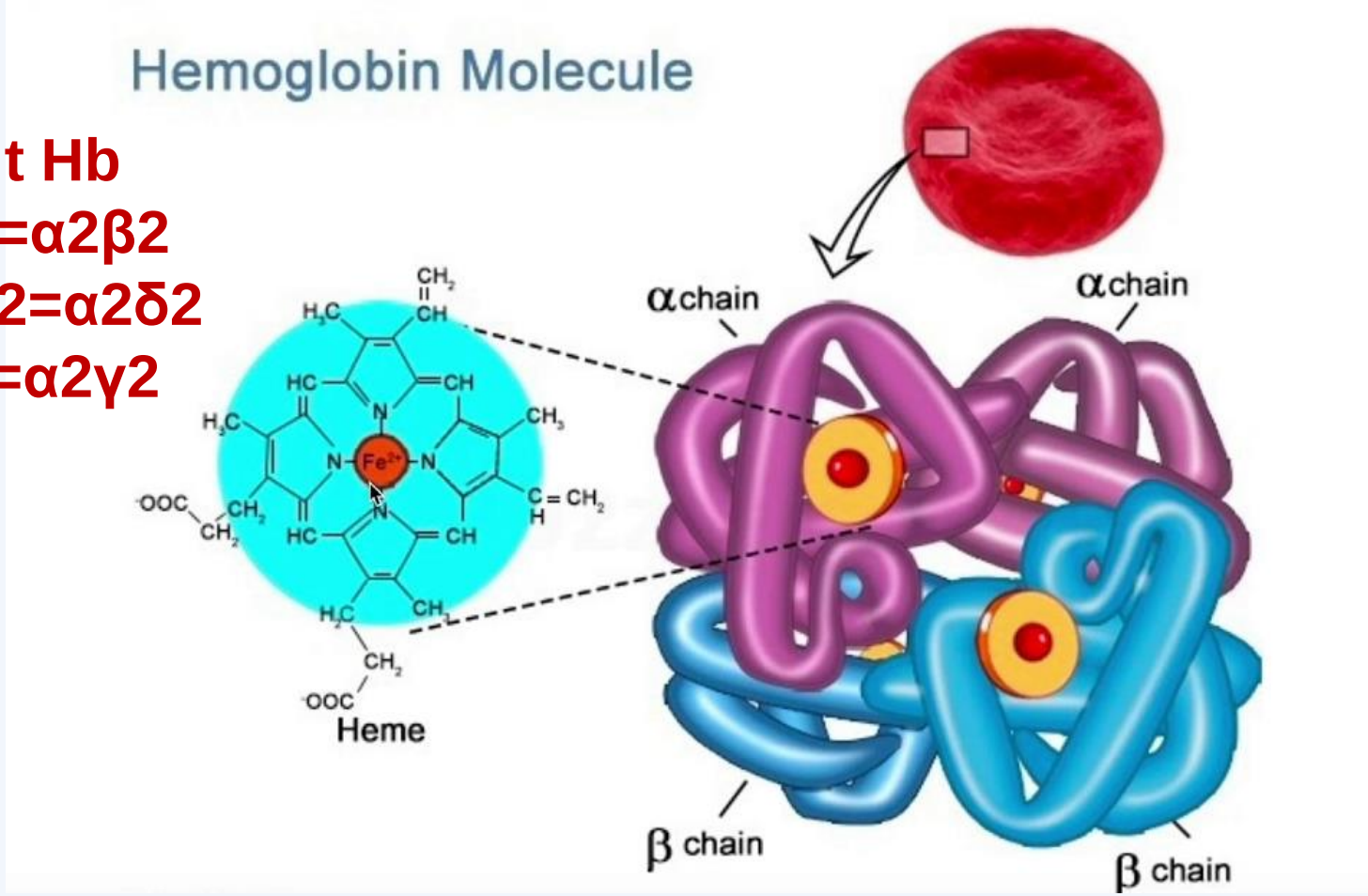
TABLE 1 Ethnic groups with a clinically significant prevalence of haemoglobins S and C and α^0 and β thalassaemia.

Haemoglobin S	African including north African, African-Caribbean, African-American, black British and any other African ethnicity (e.g. central and south American of partly African ethnicity), Greeks, southern Italians including Sicilians, Turks, Arabs, Indians
Haemoglobin C	African including African-Caribbean, African-American, Black British and any other African ethnicity (e.g. Central and South American of partly African ethnicity)
α^0 thalassaemia	Chinese, Taiwanese, Southeast Asian (Thai, Laotian, Cambodian, Vietnamese, Myanmar, Malaysian, Singaporean, Indonesian, Filipino), Cypriot, Greek, Turkish and Sardinian
β thalassaemia	All ethnic groups other than Northern Europeans

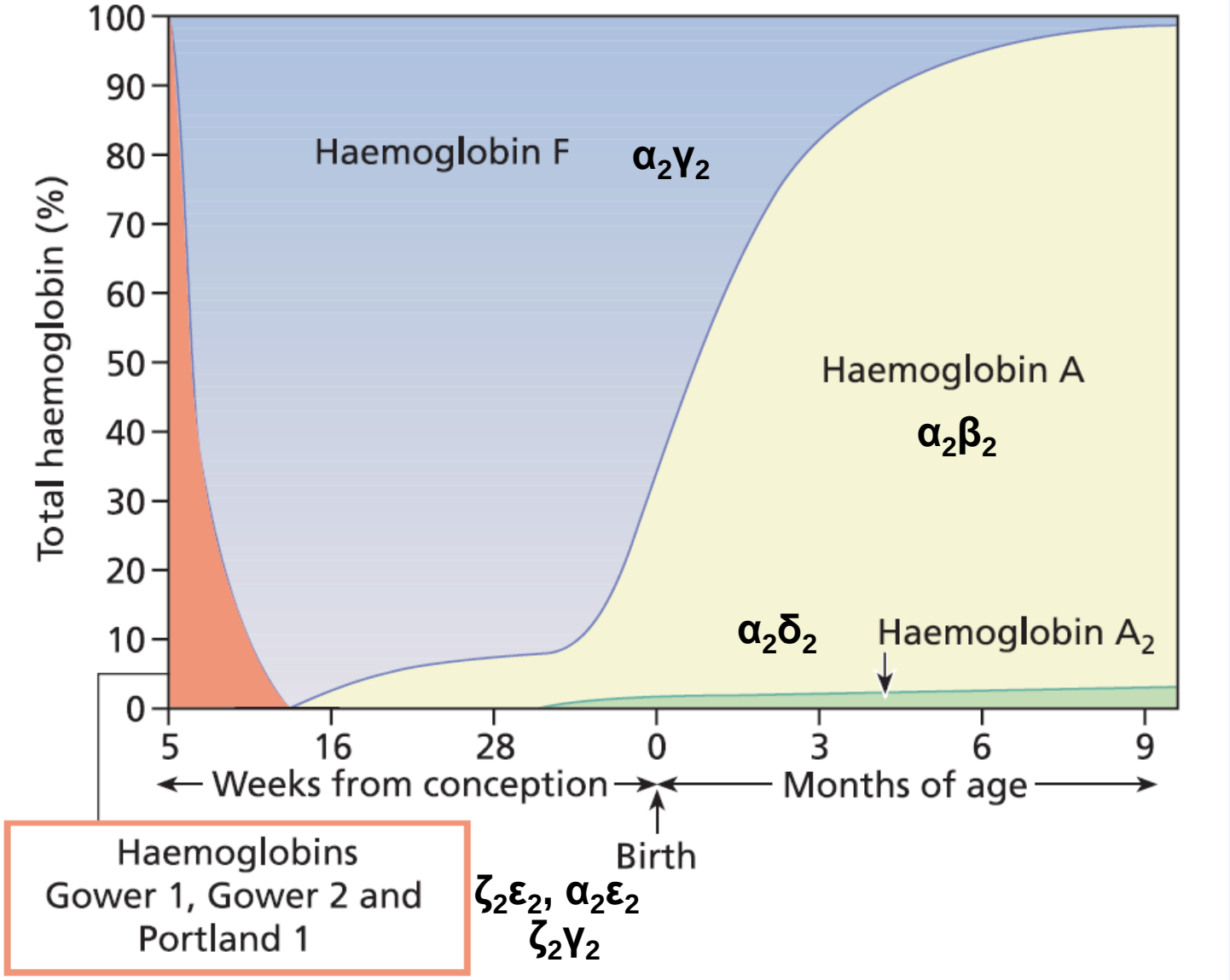
SA PATHOLOGY

Normal Haemoglobin

Adult Hb
HbA=α2β2
HbA2=α2δ2
HbF=α2γ2



SA PATHOLOGY



SA PATHOLOGY

Thalassaemia and Haemoglobinopathy
Fast facts:

- Alpha thalassaemia –reduction or absence of alpha globin chains
- Beta thalassaemia –reduction or absence of beta globin chains
- Haemoglobinopathy- an abnormal alpha OR beta globin chain (ie a genetic change that leads to a different amino acid in the alpha globin or beta globin chain) e.g. HbS, HbC, HbE
- Co-inheritance of alpha and beta variants is common (~20%)

SA PATHOLOGY

Thalassaemia and Haemoglobinopathy Fast facts:

- All have autosomal recessive inheritance (ie BOTH parents must be carriers)
- Combinations of thalassaemia (a reduction in globin chains) and haemoglobinopathy (abnormal haemoglobin chains) can give rise to a clinically significant thalassaemic syndrome

SC

S/D-Punjab

SE and E/β thalassaemia

S/O-Arab

S/Lepore and Lepore/β thalassaemia

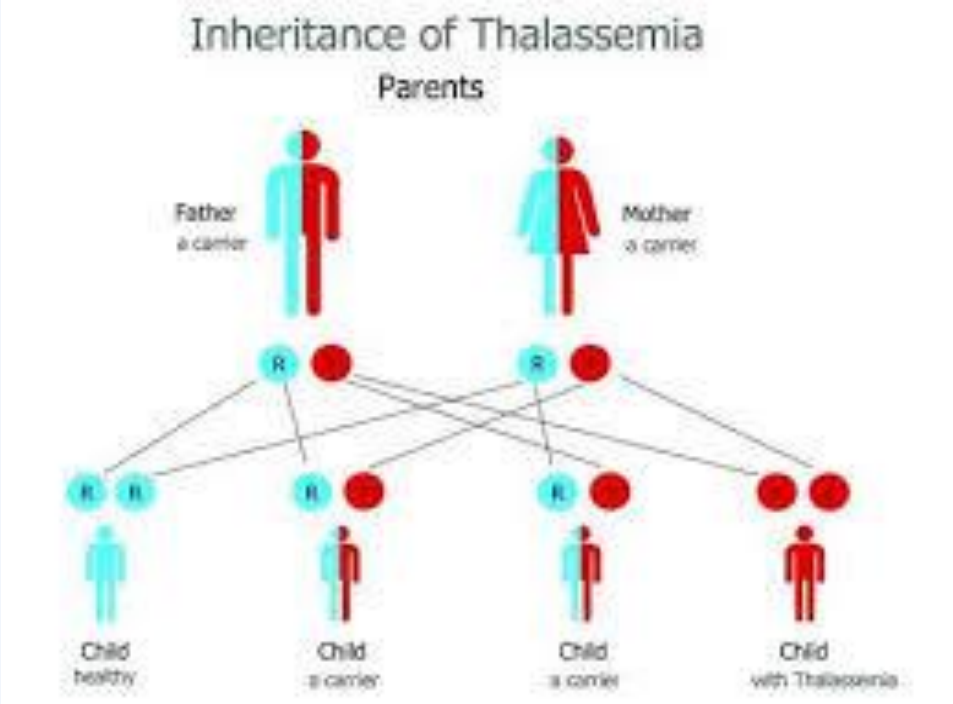
S/β thalassaemia and β thalassaemia homozygosity or compound heterozygosity

S/δβ thalassaemia and δβ thalassaemia homozygosity or compound heterozygosity with β thalassaemia

SA PATHOLOGY

Thalassaemia and Haemoglobinopathy
Fast facts:

- Two unaffected carriers of either thalassaemia or haemoglobinopathy have a ¼ risk of a fetus affected with a severe transfusion-dependent thalassaemic syndrome
  - E.g. ββ; $\alpha^0\alpha^0$; Eβ



SA PATHOLOGY

Detecting Carriers
Screening tests- first line of investigation

- Screening is based on individual risk by determining the family origin of the female and the biological father of the baby

- Complete blood examination – look at MCH, MCV, RDW, Hb, RBC count

- Iron studies – iron deficiency most common cause of microcytic, hypochromic picture

- Hb variant analysis (capillary electrophoresis or HPLC) – for detection of haemoglobinopathies, quantitation of HbA2 and HbF

ANTENATAL THALASSAEMIA/HAEMOGLOBINOPATHY SCREENING
Family of Origin Questionnaire

SA PATHOLOGY

Your family's genetic makeup may influence your child's future health. Having your family's ancestry is important to us when interpreting the findings of these tests. Please refer to the country key when answering the questions below.

Woman's details	Partner's details
Given name:	Given name:
Family name:	Family name:
Date of birth:	Date of birth:
Address:	Address:
Medicare number:	Medicare number:

Is there a known family history of thalassaemia and/or haemoglobin variant?
(e.g. sickle cell haemoglobin, haemoglobin E or C)

Woman ☐ Yes ☐ No ☐ Not sure Partner ☐ Yes ☐ No ☐ Not sure

What are your or your family's origins?

Please tick *all* boxes that apply.

	Region of family/ancestral origin	Woman	Partner
	Caucasian - United Kingdom Europe/North America/Australia	☐	☐
	Aboriginal - Torres Strait Islanders/Pacific Islands	☐	☐
	South Asian - including the Indian subcontinent	☐	☐
	Oriental - Japan, Taiwan, Korea and China	☐	☐
	Afro-Carribbean - Africa/Middle East/other African origins	☐	☐

Clinicians complete overleaf →

To be completed by Clinician

Referral to Haematology Genetic MDT

Thalassaemia/Haemoglobinopathy Pre-conceptional/Antenatal testing

This referral will not result in a clinic appointment. The MDT will review information for the couple and issue a summary letter.

Please complete both sides of this form and attach all relevant test results for both partners.

Screening test checklist	Female partner	Male partner
CBE		
Iron Studies		
Hb variant analysis		

If tests have been performed in SA Pathology copies need not be attached.

Additional relevant information or attach letter.

Copy of report to other clinicians:

Office Use ONLY	
EDC:	Consanguinous Couple ☐ Yes ☐ No
Gestation:	If yes: Relationship
UR number:	Current Pregnancy: IVF ☐ Yes ☐ No
Location: *(Hosp. Shared Services, Dr's rooms)*	If yes: Donor Egg ☐ Yes ☐ No Donor Sperm ☐ Yes ☐ No

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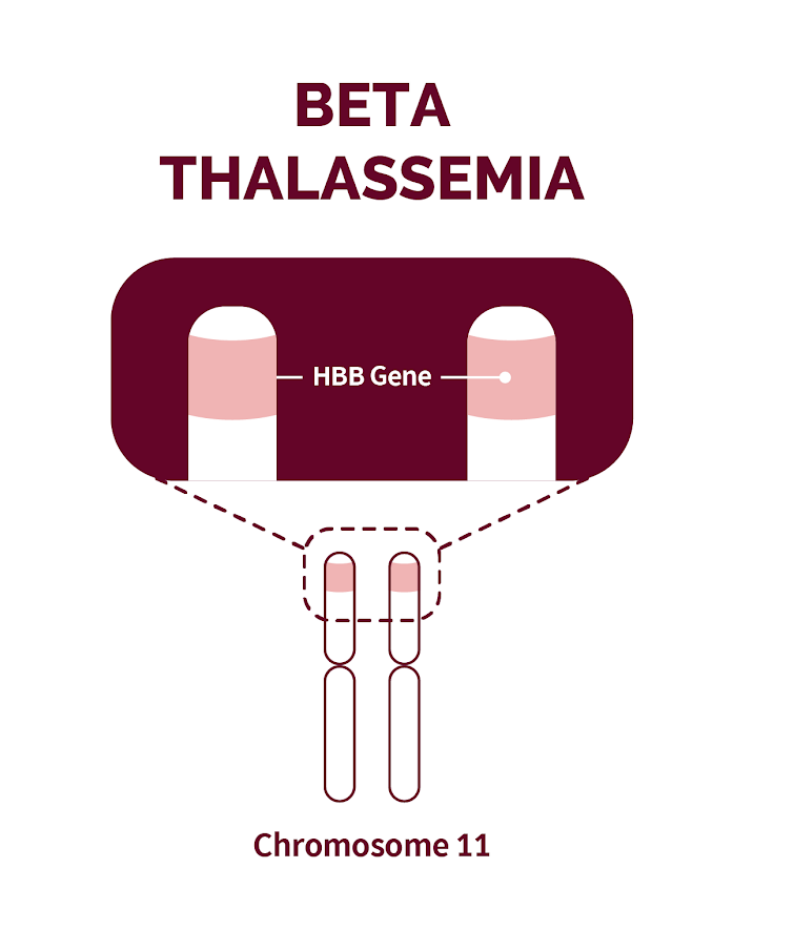
Beta Thalassaemia

- Caused by mutations- single nucleotide substitution, small deletions or insertions or rarely large deletions
- Resulting in reduced (β^{+})or absent (β^{0})production in β chains
- >350 mutations have been described
- β Thal trait- carrier
- β Thal homozygous- phenotypically variable

SA PATHOLOGY

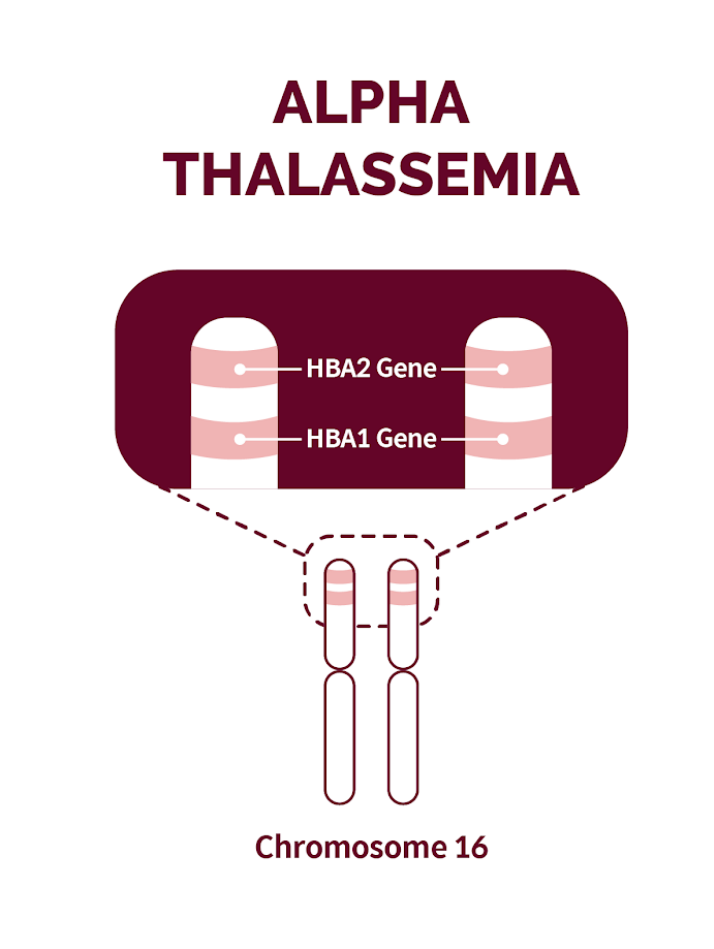
Beta Thalassaemia trait



- Low MCH <27 pg (18-28.4)
- Low MCV <80 fL (56-81.2)
- Relative increase in HbA2% (**α2δ2**) – >3.5% (normal range 1.8-3.2%)
- HbF may be slightly increased
- Note: "Silent" carriers
  - some β^+ variants have near/normal indices
  - Normal HbA2 with co-inheritance of δ gene variants or iron deficiency
- Beta thalassaemia trait masks alpha thalassaemia. Important to screen for both when assessing a couple with beta thalassaemia trait

SA PATHOLOGY

Alpha Thalassaemia



Alpha-thalassemia Genetics and Clinical Consequences

Normal	α α / α α
Carrier: Asymptomatic No abnormalities	α α / α α
α-thal minor: Asymptomatic Mild microcytic anemia	α α / α α or α α / α α
Hb H Disease: Symptomatic Hemolytic and Microcytic anemia Splenomegaly	α α / α α
Incompatible with Life Hydrops Fetalis	α α / α α

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Alpha Thalassaemia

- Deletions are the most common genetic variant
- Occasionally may be point mutations in critical regions- e.g. terminal codon leading to elongated Hb- Hb Constant Spring- non deletional forms result in more severe phenotype
- Results in reduced (α^{+}) or absent (α^{0})production of α globin

Alpha Thalassaemia trait

- Hb variable depending on how many genes affected
- MCV<79
- MCH<27
- HbA2 normal or slightly decreased
- HbF normal

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Significant Haemoglobinopathies

- All worsened by co-inheritance with beta thalassaemia:
  - HbS- single point mutation in 6th position of β globin gene- Valine replaces glutamic acid
  - HbC - single point mutation in 6th position of β globin gene- lysine replaces glutamic acid
  - HbE- single point mutation in 26th position of β globin gene- lysine replaces glutamic acid
  - HbD-Punjab-single point mutation in 121th position of β globin gene- glutamine replaces glutamic acid

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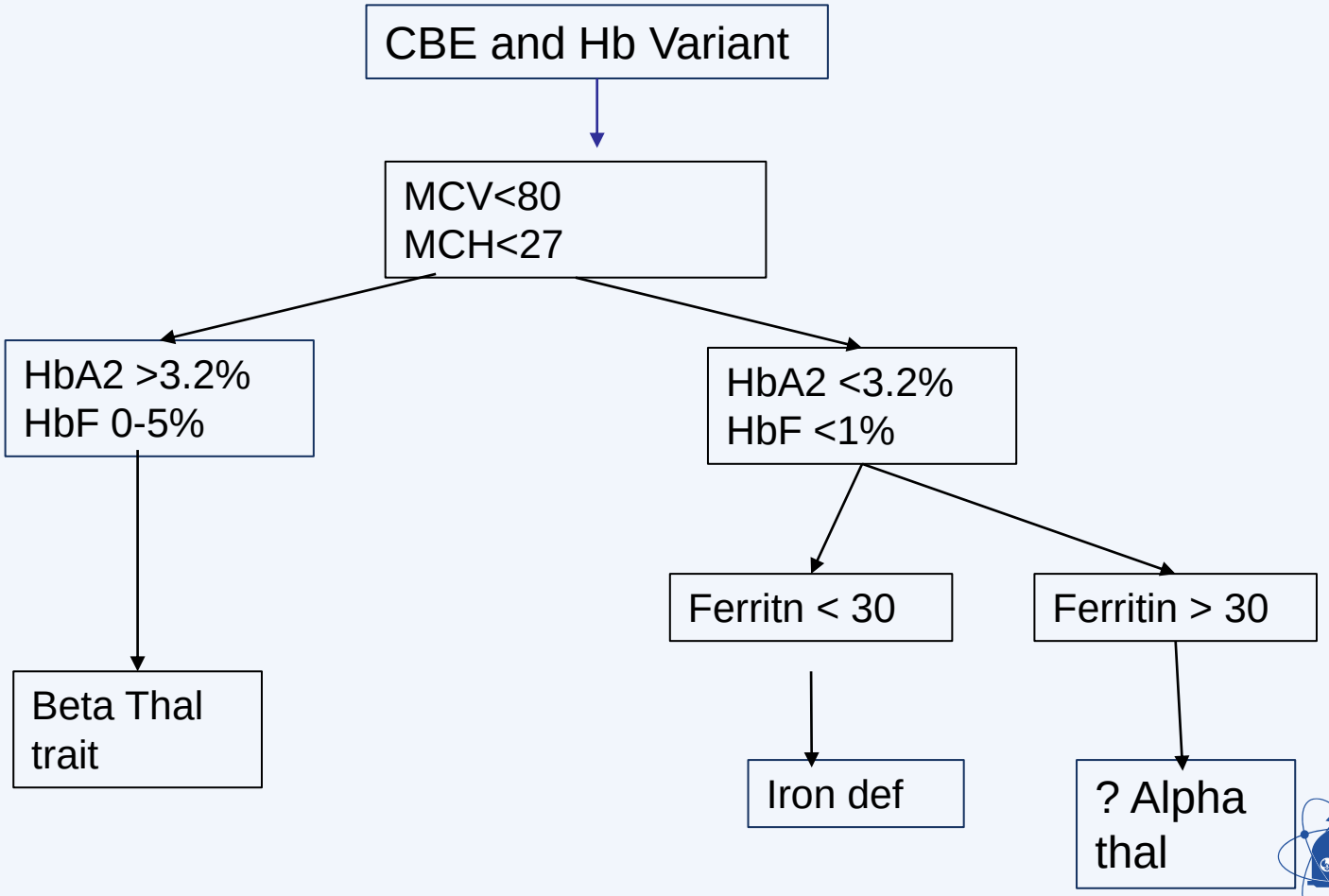
But also>1000 other variants

- Hb Lepore –reduced β-globin synthesis
- Hb C -Harlem –with HbS, sickles
- Hb O –Arab- with HbS, sickles
- Hb-Köln –highly unstable, haemolytic anaemia
- Hb-Terre Haute -extremely unstable, thalassaemic indices
- Hb M- Saskatoon
- Hb M –Hyde Park
- Hb Chesapeake
- Hb Kempsey
- And others……(Hb Little Rock; Hb Woodville)

Algorithm for Thalassaemia testing



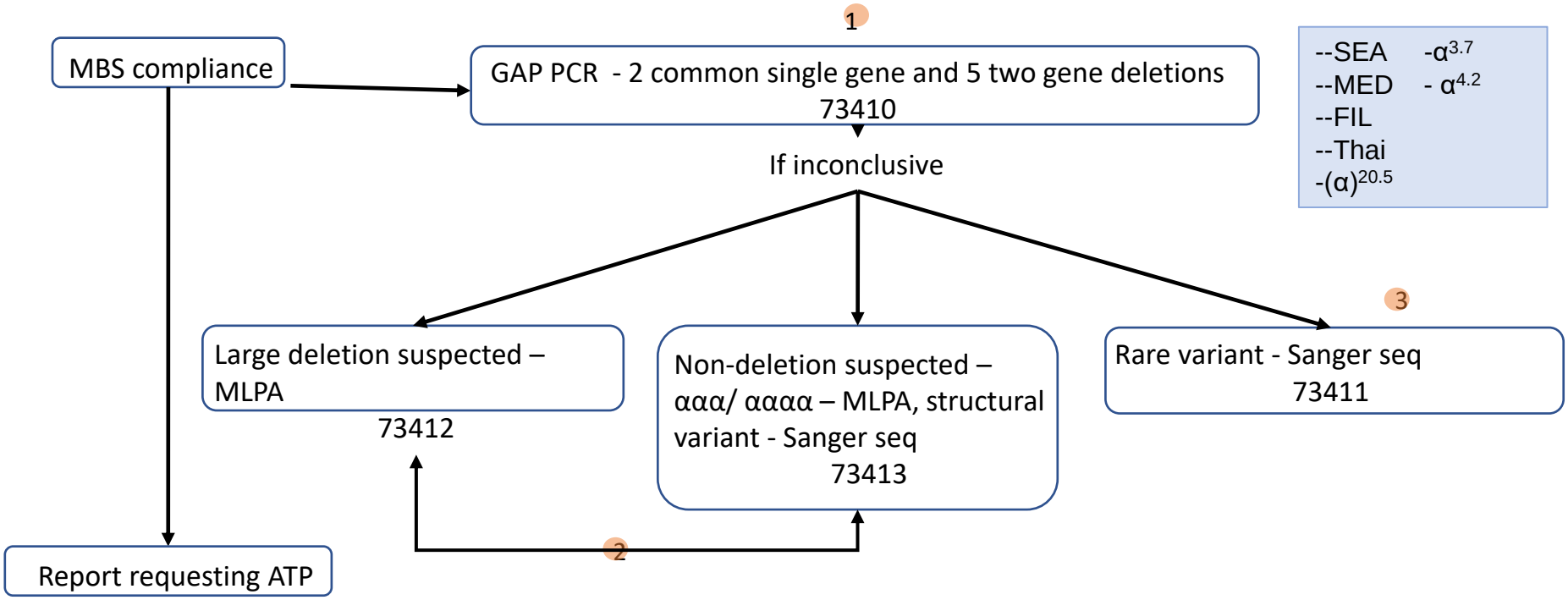
SA PATHOLOGY

Suspect alpha

MBS rebatable testing is for:

1. Diagnosis of α-thal in patients of reproductive age if patient -
   - has abnormal red cell indices + β-thal testing was not conclusive + does not have concurrent iron deficiency or is pregnant + have no historic normal cell indices.

2. Determination of carrier status in reproductive partners of patients of child=bearing potential who have been diagnosed with α-thal.



Abnormal red cell indices: MCV <80 fL, MCH <28 pg, HBA2 <3.4%

Suspect beta

- HBB screen for common variants

  - 15 common HBB gene variants in SA including HbS and HbE

  - MALDI-TOF mass spectrometry

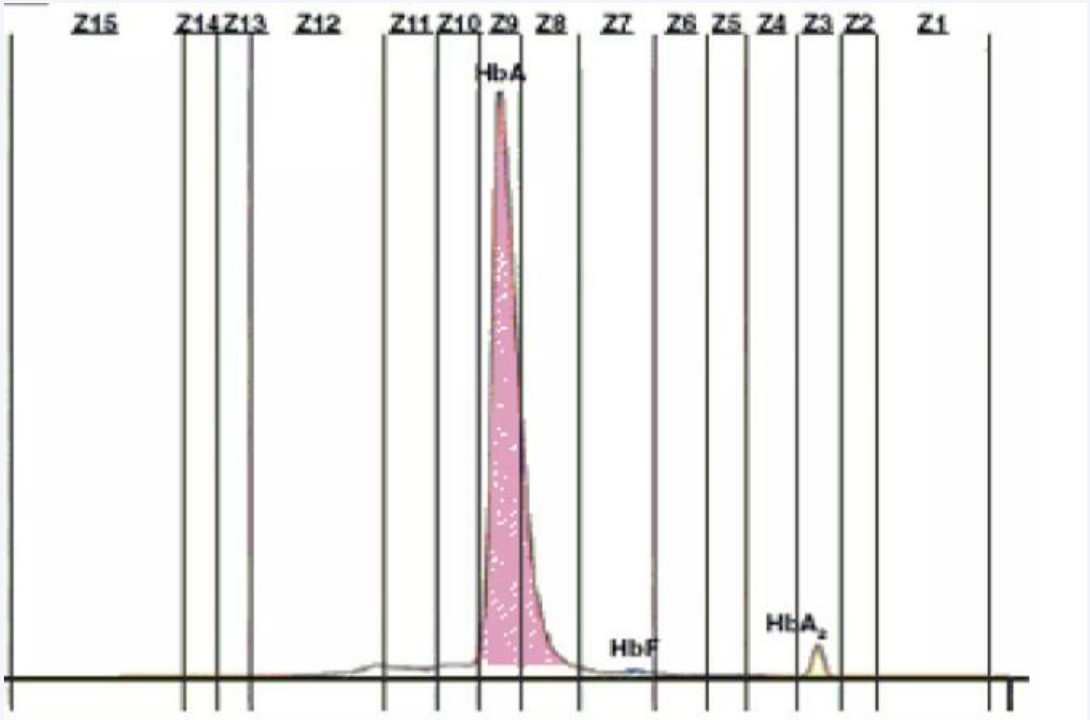
- HBB gene variant analysis

  - Allele specific PCR of a common deletion-insertion variant

- Sanger sequencing

Capillary Electrophoresis

Normal adult



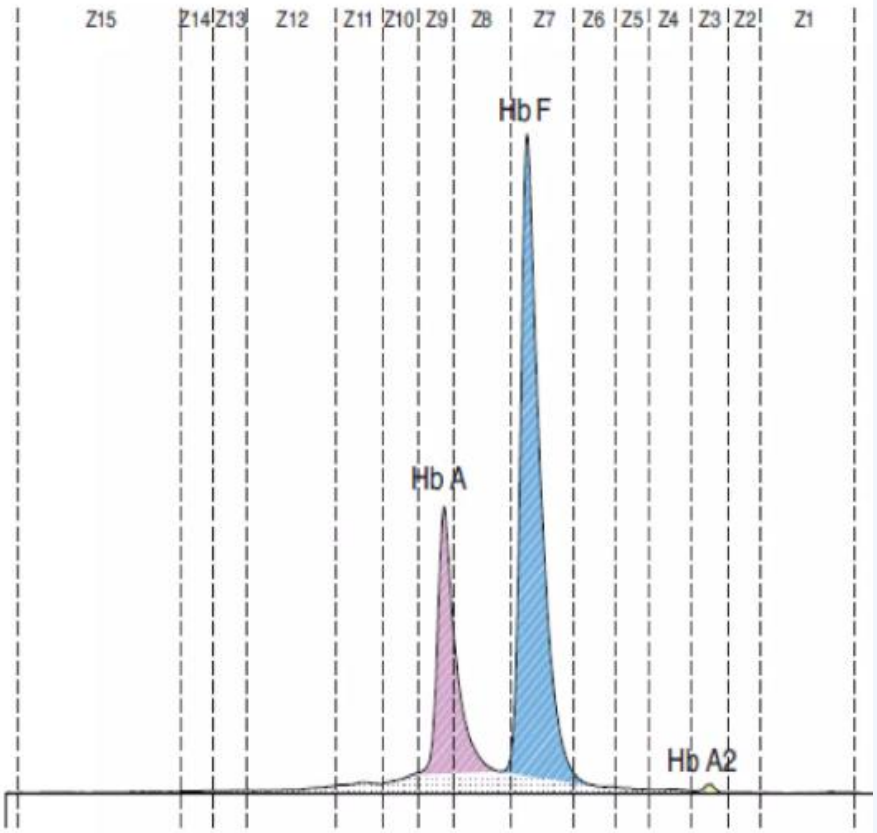
Capillary Hemoglobin Electrophoresis

Fractions	%
HbA	97.1
HbF	0.5
HbA	2.4

SA PATHOLOGY

Capillary Electrophoresis

3 week old baby



Sang de bébé (agé de 3 semaines)
Baby blood sample (3 weeks old)

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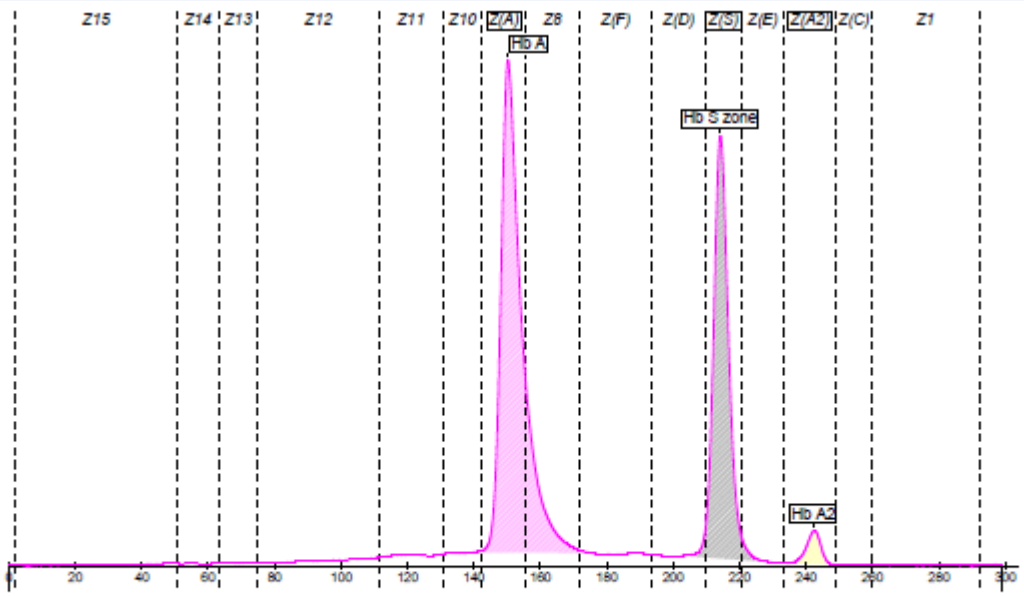
Capillary Electrophoresis

Sickle trait



Name	%	Normal Values %
Hb A	60.7	
Hb S zone	36.6	
Hb A2	2.7	

SA PATHOLOGY

CASE PRESENTATION

SA PATHOLOGY

Case 1

- Request form received
  - G5P0
  - Test requested ‘alpha thalassaemia’

SA PATHOLOGY

Case 1

Wife: Pregnant (Iron replete)

	HAEMOGLOBIN	124	g/L	(110-150)
*	R.B.C.	5.80	x10 ^12 /L	(3.50-5.00)
	P.C.V.	0.38	L/L	(0.32-0.42)
*	M.C.V.	64.7	fl	(82.0-101.0)
*	M.C.H.	21.4	pg	(27.0-31.0)
	M.C.H.C.	331	g/L	(315-355)
*	R.D.W.	16.2	%	(11.5-15.5)

Husband

*	HAEMOGLOBIN	134	g/L	(135-175)
*	R.B.C.	6.04	x10 ^12 /L	(4.50-6.00)
	P.C.V.	0.40	L/L	(0.40-0.50)
*	M.C.V.	66.4	fl	(80.0-98.0)
*	M.C.H.	22.2	pg	(27.0-33.0)
	M.C.H.C.	334	g/L	(310-360)
	R.D.W.	15.0	%	(12.0-15.0)

Case 1

Bio-Rad CDM System
DCP Variant V-II Instrument #2,

PATIENT REPORT
V2_BThal

d32643455d

Patient Data
Sample ID: 32643455
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 06/FEB/2012 12:03:01
Injection Number: 533
Run Number: 28
Rack ID: 0005
Tube Number: 5
Report Generated: 06/FEB/2012 13:10:44
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.0	0.97	849
F	0.3	---	1.07	7051
Unknown	---	1.1	1.25	23355
A2	2.8	---	3.63	69145

Suspicious of alpha trait

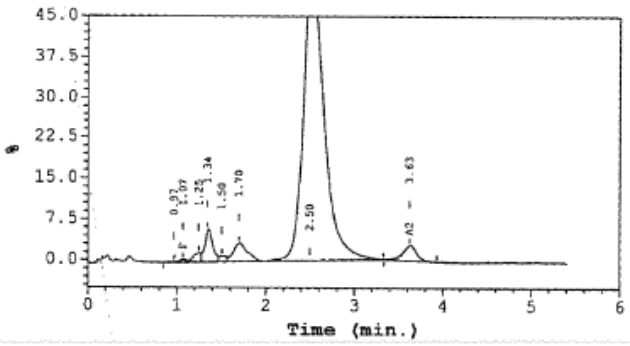
Total Area: 2,213,295

F Concentration = 0.3 %
A2 Concentration = 2.8 %

Review
MO
Validation
Database
DNA Referral

Analysis comments:

Wife



Hb =
RBC =
MCV =
MCH =
MCHC =
Plts =

Ferritin =
Iron =
Transferrin =
Trans Sat =

Sickling Test =

FAX

Bio-Rad CDM System
DCP Variant V-II Instrument #2, Serial # -11652

PATIENT REPORT
V2_BThal

Patient Data
Sample ID: 30948537
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

30948537

Analysis Data
Analysis Performed: 20/AUG/2012 13:14:51
Injection Number: 1641
Run Number: 95
Rack ID:
Tube Number: 10
Report Generated: 20/AUG/2012 15:32:39
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.96	1170
F	0.4	---	1.06	9784
Unknown	---	0.8	1.24	18477
P2	---	3.2	1.34	73389
Unknown	---	0.6	1.49	13337
P3	---	2.9	1.69	67715
Unknown	---	1.3	1.83	31365
Ao	---	69.4	2.51	1616246
A2	19.1*	---	3.74	497194

Total Area: 2,328,676

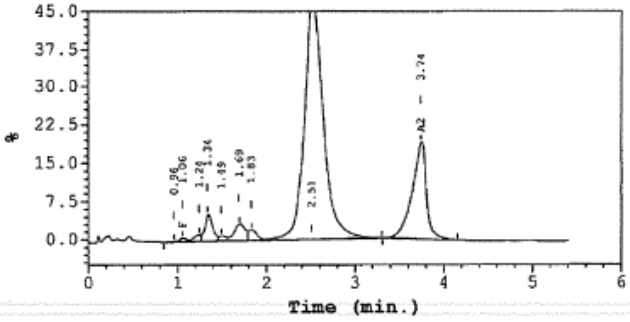
F Concentration = 0.4 %
HbE **A2 Concentration = 19.1* %**

*Values outside of expected ranges

Review
MO
Validation
Database
DNA Referral THA

Analysis comments:

Husband



Hb =
RBC =
MCV =
MCH =
MCHC =
Plts =

Ferritin =
Iron =
Transferrin =
Trans Sat =

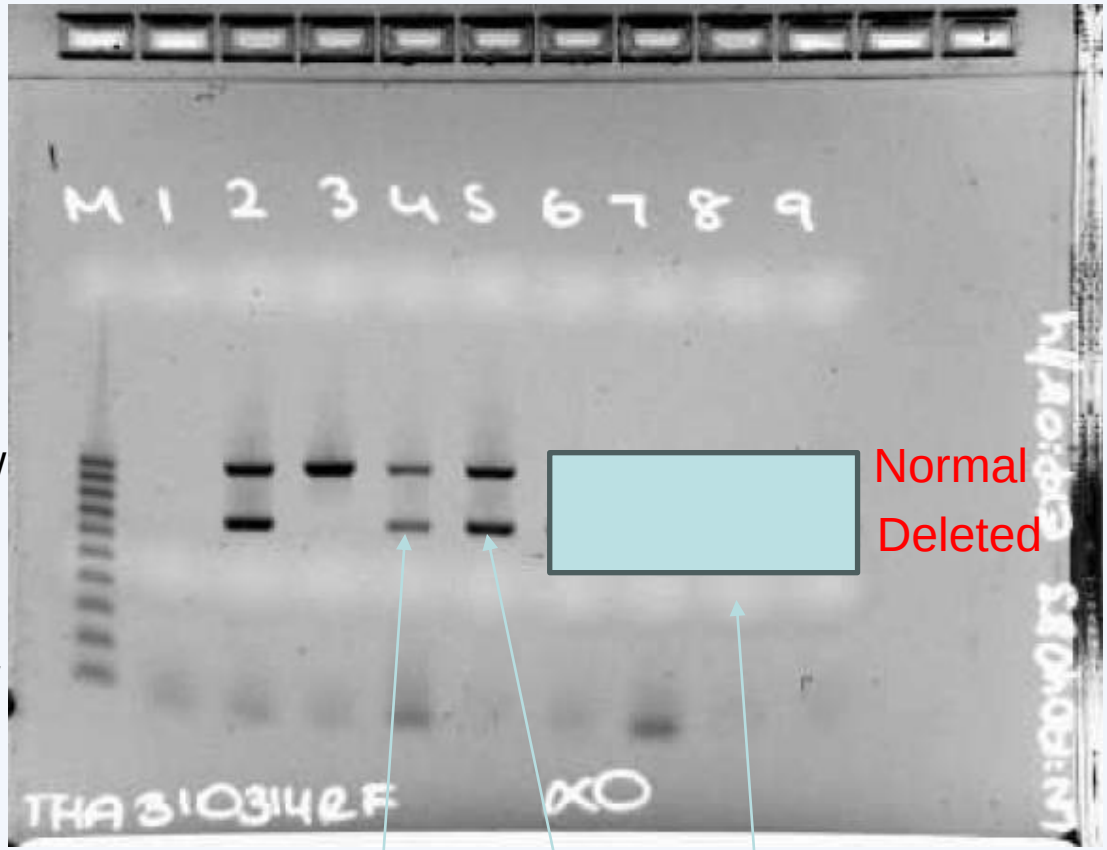
Sickling Test =

Case 1

- Previous results:
  - Male partner has previously had genetic testing and found to be a carrier of both α^0 thalassaemia ($--^{SEA}/\alpha\alpha$) and Hb E
  - If female partner also a carrier of α^0 $--^{SEA}$ thalassaemia
  - Couple therefore has a ¼ risk of Bart's hydrops fetalis
  - Reproductive significance of this finding had not been appreciated

SA PATHOLOGY

GAP PCR Results - Prenatal Diagnosis



M- Molecular Weight marker

1- Negative control

2- SEA control

3- Normal control

4- Mother SEA heterozygote

5-Father SEA heterozygote

6-8 Fetus- SEA homozygote (Barts hydrops fetalis- no functional haemoglobin alpha genes)

9- Control for SEA homozygous

SA PATHOLOGY

Case 2

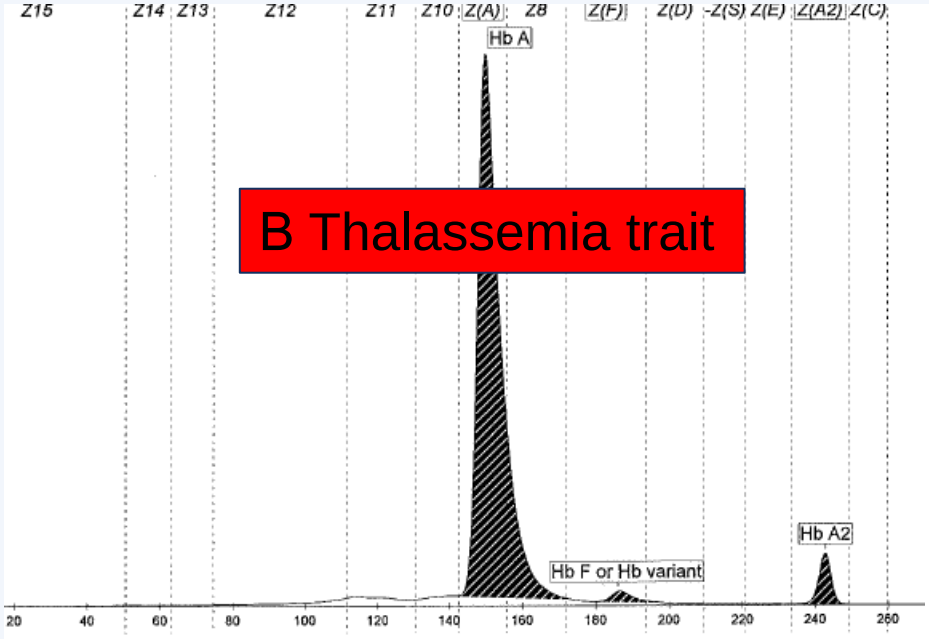
- 32 yo female, approx. 22 weeks pregnant
  - Hb 97
  - RCC 4.49
  - MCV 66.8
  - MCH 22
  - RDW 7.64

- 33 yr old male partner
  - Hb 132
  - normal indices

SA PATHOLOGY

Case 2

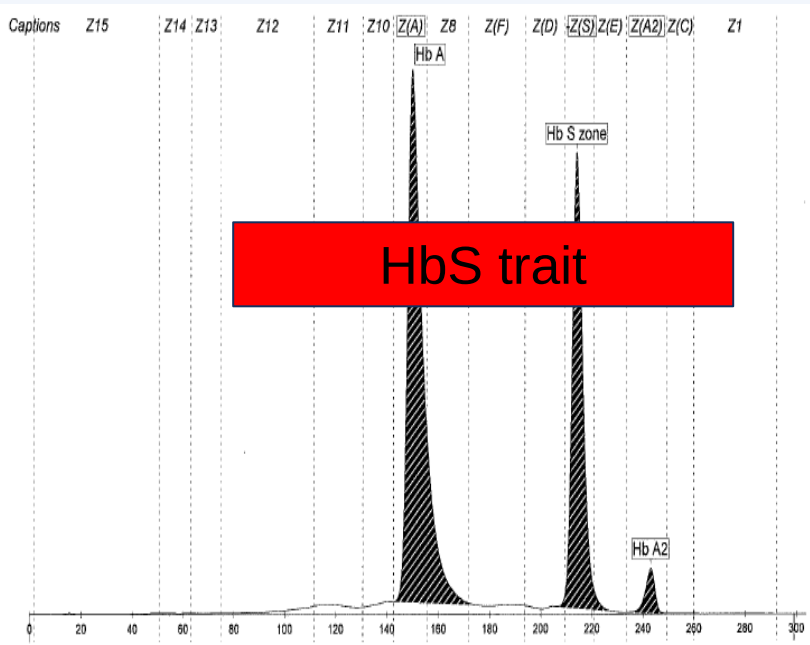
Female



B Thalassemia trait

Name	%		Normal Values %
Hb A	89.2	<	96.8 - 98.2
Hb F or Hb variant	4.7	>	=< 1.0
Hb A2	6.1	>	1.8 - 3.2

Male



HbS trait

Name	%	Normal Values %
Hb A	59.7	
Hb S zone	37.1	
Hb A2	3.2	

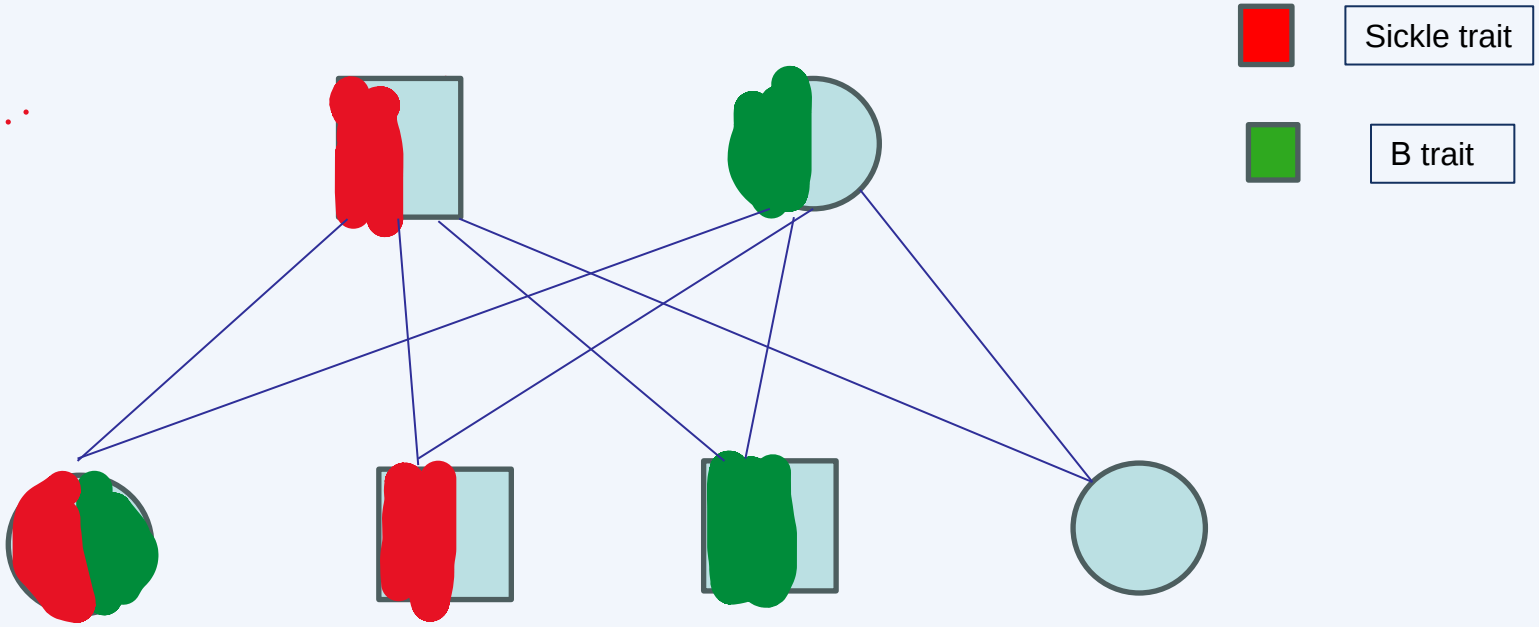
SA PATHOLOGY

Case 2

- Female
  - HBB common variant screen
    - Carrier of beta+ thalassaemia
    - 1:2 chance offspring will inherit this variant
- Male
  - HBB common variant screen
    - HbS trait

SA PATHOLOGY

Case 2



Couple have 1 in 4 chances of offspring with HbSβ+

SA PATHOLOGY

Summary

- All women should be tested for thalassaemia and haemoglobinopathy before or early in pregnancy
  - CBE
  - Fe studies
  - Hb variant analysis
- For at-risk couples (based on family history, ethnicity, or maternal results) partner testing should also be performed
- Cost of testing every pregnant woman in the state would be offset by preventing a single case of thalassaemia major

SA PATHOLOGY

Caveat

- May not detect silent β thal carriers
- May miss combined α and β thal carriers
- May not detect coinheritance of β thal and α triplication
- May miss Hb variants in non high risk groups
- May not detect Thal carriers if MCV/MCH raised by folate/Vit B12 def, liver disease, etc

SA PATHOLOGY

SA PATHOLOGY