DIAGNOSIS OF THE THALASSAEMIA SYNDROMES:

MEASUREMENT OF HAEMOGLOBIN $A_2$

Barbara Wild

UK National External Quality Assessment Scheme
London
Globin biosynthesis

<table>
<thead>
<tr>
<th>%</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td></td>
<td>3</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ</td>
<td>3</td>
<td></td>
<td></td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>ζ + ε</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>δ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Hb F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Birth
The importance of Hb A\textsubscript{2} measurement

Hb A\textsubscript{2} is measured as a \textit{proportion} of the \textit{total} haemoglobins present, not as an absolute amount.

Hb A\textsubscript{2} measurement is used as a marker for beta thalassaemia trait. Carrier detection is important because:

Beta thalassaemia carriers are asymptomatic but homozygous beta thalassaemia is a life-threatening disorder.
The importance of Hb A$_2$ measurement

- Accurate and reliable measurement of Hb A$_2$ is essential for the diagnosis of beta thalassaemia trait
- Small difference (if any) between normal & abnormal levels

- Antenatal women should be screened for beta thalassaemia trait
- Carriers: recommend partner testing
- Prediction of genetic risk

- Failure to detect condition may result in newborn with a medically significant condition
Screening for beta thalassaemia trait

- Full blood count with red cell indices: RBC, Mean Cell Volume and Mean Cell Haemoglobin
- Hb A₂ %
- Hb F %
- Screen for haemoglobin variants
- Iron status - ferritin, zinc protoporphyrin
- Family history
Measurement of Hb A₂

Automated methods
• High Performance Liquid Chromatography
• Capillary electrophoresis
• Mass spectrometry

Manual methods
• Hb electrophoresis with elution
• Microcolumn chromatography

Interpretation
Normal: 2.2-3.5% (usually <3.3%)
Beta thalassaemia trait: >3.5%
High performance liquid chromatography

General principle

- Utilises a weak cation-exchange column
- Hb molecules adsorb onto the column saturated with low ionic strength buffer
- Buffer with increased ionic strength used to elute haemoglobins from column
- Haemoglobins will elute when ionic strength of eluting solution exceeds that of the haemoglobins
- Retention time of a particular haemoglobin is characteristic and reproducible, but not unique
### HPLC analysis - normal adult

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.1</td>
<td>0.59</td>
<td>3217</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.1</td>
<td>1.00</td>
<td>3321</td>
</tr>
<tr>
<td>F</td>
<td>0.3</td>
<td>---</td>
<td>1.10</td>
<td>7753</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>1.1</td>
<td>1.21</td>
<td>29205</td>
</tr>
<tr>
<td>P2</td>
<td>---</td>
<td>4.8</td>
<td>1.29</td>
<td>125635</td>
</tr>
<tr>
<td>P3</td>
<td>---</td>
<td>4.7</td>
<td>1.65</td>
<td>121990</td>
</tr>
<tr>
<td>Ao</td>
<td>---</td>
<td>86.0</td>
<td>2.33</td>
<td>2232443</td>
</tr>
<tr>
<td>A2</td>
<td>3.1</td>
<td>---</td>
<td>3.59</td>
<td>70883</td>
</tr>
</tbody>
</table>

Total Area: 2594456

**F Concentration** = 0.3 %  
**A2 Concentration** = 3.1 %

**Analysis comments:**
Beta thalassaemia trait

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.1</td>
<td>0.60</td>
<td>2439</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.1</td>
<td>0.98</td>
<td>2463</td>
</tr>
<tr>
<td>F</td>
<td>1.0</td>
<td>---</td>
<td>1.11</td>
<td>23374</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>1.0</td>
<td>1.22</td>
<td>26601</td>
</tr>
<tr>
<td>F2</td>
<td>---</td>
<td>5.0</td>
<td>1.29</td>
<td>120590</td>
</tr>
<tr>
<td>F3</td>
<td>---</td>
<td>5.2</td>
<td>1.67</td>
<td>133229</td>
</tr>
<tr>
<td>A0</td>
<td>---</td>
<td>83.0</td>
<td>2.34</td>
<td>2115341</td>
</tr>
<tr>
<td>A2</td>
<td>5.2*</td>
<td>---</td>
<td>3.61</td>
<td>115816</td>
</tr>
</tbody>
</table>

Total Area: 2547853

F Concentration = 1.0 %
A2 Concentration = 5.2* %

*Values outside of expected ranges

Analysis comments:
Sickle cell trait

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>0.6</td>
<td>---</td>
<td>1.19</td>
<td>16136</td>
</tr>
<tr>
<td>P2</td>
<td>---</td>
<td>3.8</td>
<td>1.27</td>
<td>106506</td>
</tr>
<tr>
<td>P3</td>
<td>---</td>
<td>2.8</td>
<td>1.68</td>
<td>78920</td>
</tr>
<tr>
<td>Ac</td>
<td>---</td>
<td>54.8</td>
<td>2.39</td>
<td>1539681</td>
</tr>
<tr>
<td>A2</td>
<td>2.9</td>
<td>---</td>
<td>3.01</td>
<td>75289</td>
</tr>
<tr>
<td>S-window</td>
<td>---</td>
<td>35.3</td>
<td>4.51</td>
<td>991769</td>
</tr>
</tbody>
</table>

Total Area: 2808300

F Concentration = 0.6 %
A2 Concentration = 2.9 %

Analysis comments:
Hb $\beta^+$thalassaemia

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area</th>
<th>% Area</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>9.0*</td>
<td>---</td>
<td>1.11</td>
<td>153554</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.7</td>
<td>2.11</td>
<td>11443</td>
</tr>
<tr>
<td>Ao</td>
<td>---</td>
<td>16.2</td>
<td>2.47</td>
<td>273375</td>
</tr>
<tr>
<td>A2</td>
<td>6.5*</td>
<td>---</td>
<td>3.62</td>
<td>99298</td>
</tr>
<tr>
<td>S-window</td>
<td>---</td>
<td>68.1</td>
<td>4.50</td>
<td>1149258</td>
</tr>
</tbody>
</table>

Total Area: 1686929

F Concentration = 9.0* %
A2 Concentration = 6.5* %

*Values outside of expected ranges

Analysis comments:
δ chain variant

Consider total Hb A₂ and review red cell indices

Note: also check for carry-over
Hb Lepore trait

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>1.5</td>
<td>---</td>
<td>1.07</td>
<td>25436</td>
</tr>
<tr>
<td>Unknown</td>
<td>---</td>
<td>0.9</td>
<td>1.22</td>
<td>16792</td>
</tr>
<tr>
<td>P2</td>
<td>---</td>
<td>3.7</td>
<td>1.29</td>
<td>71547</td>
</tr>
<tr>
<td>P3</td>
<td>---</td>
<td>4.6</td>
<td>1.63</td>
<td>88564</td>
</tr>
<tr>
<td>Ao</td>
<td>---</td>
<td>78.0</td>
<td>2.47</td>
<td>1518633</td>
</tr>
<tr>
<td>A2</td>
<td>12.2*</td>
<td>---</td>
<td>3.51</td>
<td>225141</td>
</tr>
</tbody>
</table>

Total Area: 1946114

F Concentration = 1.5 %
A2 Concentration = 12.2* %

*Values outside of expected ranges

Analysis comments:
Hb Kenya trait

Notes

X = Haemoglobin Kenya
(+ A2 on HPLC)

Y on IEF is unidentified
No known clinical significance

Haemoglobin Kenya heterozygotes have increased haemoglobin F (typically around 11%)
The percentage of haemoglobin Kenya is usually higher than in this patient (reported as 15-18%)

Microcytosis and hypochromia are often present

Haemoglobin Kenya (γβ fusion) heterozygote
Hb Fort Worth trait

Haemoglobin Fort Worth - $\alpha 27$ (Glu $\rightarrow$ Gly) heterozygote
Capillary electrophoresis

• Utilises a thin capillary of silica, diameter approx 50-75 μm
• Inner surface of the capillary has a negative charge
• High voltage applied (10-30kv) – capillary generates endo-osmotic flow (EOF) towards cathode
• Hbs separated because of different charges-fractions move towards the cathode because of EOF

• Electropherograms of peaks of a particular haemoglobin is characteristic and reproducible, *but not unique*
Figure 1: Normal sample

Figure 2: AFSC control

Figure 3: Heterozygous A/S

Figure 4: Alpha thalassemia with Hb Bart's

The CAPILLARY™ Hemoglobin assay
Capillary electrophoresis

Haemoglobin Electrophoresis

<table>
<thead>
<tr>
<th>Name</th>
<th>%</th>
<th>Normal Values %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A</td>
<td>94.4</td>
<td>&lt;</td>
</tr>
<tr>
<td>Hb F or Hb variant</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Hb A2</td>
<td>5.2</td>
<td>&gt;</td>
</tr>
</tbody>
</table>
High throughput haemoglobin variant mutation analysis and protein biomarker quantitation using dried blood spots

Neil Dalton, Charles Turner & Yvonne Daniel
The use of Mass Spectrometry for screening and identification of the haemoglobinopathies

- MS technique based on mass differences in globin chains
- Initially used for identification of variants detected on screening
- Being developed as potential approach for haemoglobinopathy screening
ESI-MS: normal whole blood

Original spectrum

Deconvoluted spectra
Electrospray ionisation mass spectrometry
Hb Johnstown

Patient FP

15126.6

15867.4

+14.1
High throughput haemoglobin variant mutation analysis and protein biomarker quantitation using dried blood spots

Wild-type T1 VHLTPEEK
MW 951.5

Doubly charged peptide, m/z 476.8
Product ion (y4), m/z 502.3

Sickle T1 VHLTPVEK
MW 921.5

Doubly charged peptide, m/z 461.8
Product ion (y4), m/z 472.5
Wild-type βT1 isolation
Sickle βT1 isolation
High throughput haemoglobin variant mutation analysis and protein biomarker quantitation using dried blood spots

Protein/peptide quantitation: Antenatal screening for \( \beta \)-thalassaemia trait

HbA\(_2\) is about 2\% of total haemoglobin

4\% in \( \beta \)-thalassaemia trait

HbA is \( \alpha_2\beta_2 \)

HbA\(_2\) is \( \alpha_2\delta_2 \)

Could the \( \delta/\beta \) ratio be used as a biomarker for \( \beta \)-thalassaemia trait?

What are the differences in the peptides?
<table>
<thead>
<tr>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Delta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T4</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Beta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu-Leu-Val-Tyr-Pro-Trp-Thr-Gln-Arg</td>
<td>Phe-Phe-Glu-Ser-Phe-Gly-Asp-Leu-Ser-Thr-Pro-Asp-Ala-Val-Met-Gly-Asn-Pro-Lys</td>
<td>Val-Lys</td>
</tr>
<tr>
<td><strong>Delta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu-Leu-Val-Tyr-Pro-Trp-Thr-Gln-Arg</td>
<td>Phe-Phe-Glu-Ser-Phe-Gly-Asp-Leu-Ser-Ser-Pro-Asp-Ala-Val-Met-Gly-Asn-Pro-Lys</td>
<td>Val-Lys</td>
</tr>
<tr>
<td><strong>T7</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Beta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Delta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T10</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Beta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Delta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T13</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Beta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu-Phe-Thr-Pro-Pro-Val-Gln-Ala-Ala-Tyr-Gln-Lys</td>
<td>Val-Val-Ala-Gly-Val-Ala-Asn-Ala-Leu-Ala-His-Lys</td>
<td>Tyr-His</td>
</tr>
<tr>
<td><strong>Delta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T16</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Delta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyr-His</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Measurement of $\delta:\beta$ globin peptide ratio

- Samples subjected to tryptic digestion
- Multiple Reaction Monitoring undertaken for
  - $\delta$ T2, T3 and T14 peptides
  - $\beta$ T2, T3 and T13 peptides
  $\delta:\beta$ peptide ratios calculated

Study validated the quantitative $\delta:\beta$ globin peptide ratio as a surrogate marker of Hb A$_2$

Developed within concept of National Screening Programme needs

Daniel et al 2007
Interpretation of Hb A₂ levels

Hb A₂ percentage is increased in:

- Beta thalassaemia trait
- Presence of an unstable haemoglobin
- Hyperthyroidism
- Some cases of congenital dyserythropoietic anaemia, type I
- HIV infection

- Sickle cell trait or anaemia
HPLC analysis – sickle cell trait

Normal FBC

Hb S% : 35-45

Hb A₂ may be raised
Hb Yokohama trait

FA Dad SF Dad AC RB AS RB

RB Dad AFSE RB Dad AA

RB AS RB AC Dad SF Dad FA

F Concentration = 10.3* %
A2 Concentration = 4.2* %

*Values outside of expected ranges

Analysis comments:
Interpretation of Hb A$_2$ values

Haemoglobin A$_2$ percentage is decreased in
- δ thalassaemia
- Delta/beta thalassaemia
- α thalassaemia trait or haemoglobin H disease
- Severe iron deficiency
National Sickle & Thalassaemia Screening Programme

• Established to provide a linked screening programme for antenatal women and newborn
• Universal screening
• Established laboratory standards
• Standardised reporting formats
• Standardised methodology (newborn)

• Decision algorithm (antenatal)
NSC&TSP: High Prevalence Screening

FBC and HPLC

Hb Variant
- HbS, HbC, HbD, HbE, Hb O<sub>Arab</sub>, Hb Lepore
  - Test partner
- Other variant
  - refer to Consultant Haematologist*

No variant

MCH < 27
- HbA2 >= 3.5 beta thal trait
  - Test partner
- HbA2 < 3.5
  - MCH < 25
    - Consider ethnic group
      - High risk of alpha zero thalassaemia**
        - Test partner
        - No further action
      - low risk of alpha zero thalassaemia**
        - No further action
  - MCH >= 25
    - Iron deficiency alpha thal
      - Test partner

MCH >= 27
- HbA2 > 4.0 or HbF > 5%
  - refer to Consultant Haematologist*
- HbA2 <= 4.0 HbF <= 5%
  - No further action***

Test partner
From: Haemoglobinopathy diagnosis, BJ Bain

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Origin</th>
<th>Usual mean Hb A₂ (%)</th>
<th>Usual mean MCH (pg)</th>
<th>Usual mean MCV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent β thalassaemia trait (normal MCV, MCH, and Hb A₂ %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−101 (C → T)</td>
<td>Mediterranean</td>
<td>3.3</td>
<td>28</td>
<td>85</td>
</tr>
<tr>
<td>−92 (C → T)</td>
<td>Mediterranean</td>
<td>3.5</td>
<td>28</td>
<td>82</td>
</tr>
<tr>
<td>IVSII-844 (C → G)</td>
<td>Mediterranean (Italian)</td>
<td>3.5</td>
<td></td>
<td>85</td>
</tr>
<tr>
<td>+33 C → G [64]</td>
<td>Mediterranean (Greek, Cypriot)</td>
<td>3.0</td>
<td>29</td>
<td>86</td>
</tr>
<tr>
<td>+10 (−T) [65]</td>
<td>Mediterranean (Greek, one case)</td>
<td>2.6</td>
<td>32</td>
<td>97</td>
</tr>
<tr>
<td>+1480 C → G (termination codon +6 C → G)</td>
<td>Mediterranean (Greek)</td>
<td>2.7 [62]</td>
<td>28</td>
<td>88</td>
</tr>
<tr>
<td>Almost silent β thalassaemia trait (reduced MCV, MCH, normal Hb A₂ %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSI-6 (T → C)</td>
<td>Mediterranean*</td>
<td>3.5</td>
<td>23</td>
<td>71</td>
</tr>
<tr>
<td>Codon 27 (G → T) (haemoglobin Knossos†)</td>
<td>Mediterranean and Middle Eastern</td>
<td>2.1</td>
<td>25</td>
<td>71</td>
</tr>
<tr>
<td>IVSI-5 (G → A) Corfu δβ‡</td>
<td>Mediterranean</td>
<td>3.5</td>
<td>25</td>
<td>70</td>
</tr>
<tr>
<td>IVSI-128 (T → G)</td>
<td>Saudi</td>
<td>3.4</td>
<td>25</td>
<td>80</td>
</tr>
<tr>
<td>CAP +1 (A → C)</td>
<td>South Asian Italian</td>
<td>1.6§</td>
<td>23.5§</td>
<td>76§</td>
</tr>
<tr>
<td>Mutation not linked to β globin gene cluster [43]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+22 G → A [66]</td>
<td>Turkish, Bulgarian</td>
<td>3.9</td>
<td>23.5</td>
<td>79</td>
</tr>
<tr>
<td>Indices typical of thalassaemia trait but Hb A₂ % normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β Thalassaemia caused by deletion of the locus control region</td>
<td>Various</td>
<td>Normal</td>
<td>Typical of β thalassaemia</td>
<td>Typical of β thalassaemia</td>
</tr>
<tr>
<td>γδβ Thalassaemia</td>
<td>Various</td>
<td>Normal</td>
<td>Typical of β thalassaemia</td>
<td>Typical of β thalassaemia</td>
</tr>
</tbody>
</table>
Risk assessment:
UK National screening programme

• The following conditions will be missed:
• Silent or near silent beta thalassaemia carrier
• Possible beta thalassaemia carrier obscured by severe iron deficiency
• Alpha zero thalassaemia occurring outside of the defined at-risk family origins
• Dominant haemoglobinopathies where the woman has no haemoglobinopathy
• Any significant variant not detected by HPLC
Normal Hb A$_2$ $\beta$ thalassaemia in Europe

**Aim:** To determine the extent of the problem associated with normal Hb A$_2$ $\beta$ thalassaemia mutations

**Subjects:** 226 patients from Tunisia, Greece, Cyprus and UK

**Criteria for selection:** Hb A$_2$ values of 3.3-3.8%

**Methods:** Samples analysed by ARMS-PCR & $\beta$ sequencing
Normal Hb A\textsubscript{2} $\beta$ thalassaemia in Europe

• 22 cases were outside of the ‘average’ A\textsubscript{2} and MCH groups
  Of these:
    All of the IVS1-6 patients had a reduced MCH
    10/13 of the CAP+1 patients had a reduced MCH

• An additional 35 patients with Hb A\textsubscript{2} values >3.5% gave normal $\beta$ gene sequencing results
Hb A$_2$ values of a standard $\beta$-thalassaemia mutation (IVSI-5 G→C): 4.5% - 6.5%
Hb A$_2$ values of an atypical $\beta$-thalassaemia mutation (CAP+1 A $\rightarrow$ C)
# Average values

<table>
<thead>
<tr>
<th>mutation</th>
<th>cases</th>
<th>Hb A₂</th>
<th>MCH</th>
<th>MCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>+1480 (C→G)</td>
<td>18</td>
<td>2.9</td>
<td>28.2</td>
<td>89</td>
</tr>
<tr>
<td>-101 (C→T)</td>
<td>42</td>
<td>3.8</td>
<td>29.0</td>
<td>89</td>
</tr>
<tr>
<td>CAP+1 (A→C)</td>
<td>75</td>
<td>3.7</td>
<td>25.4</td>
<td>79</td>
</tr>
<tr>
<td>IVSI-6 (T→C)</td>
<td>34</td>
<td>4.2</td>
<td>22.7</td>
<td>72</td>
</tr>
<tr>
<td>Poly A (A→G)</td>
<td>10</td>
<td>3.9</td>
<td>24.7</td>
<td>76</td>
</tr>
<tr>
<td>Poly A (T→C)</td>
<td>5</td>
<td>4.0</td>
<td>22.4</td>
<td>73</td>
</tr>
<tr>
<td>Poly A (-AT)</td>
<td>2</td>
<td>3.8</td>
<td>22.7</td>
<td>72</td>
</tr>
<tr>
<td>Poly A (-AA)</td>
<td>8</td>
<td>4.0</td>
<td>23.6</td>
<td>73</td>
</tr>
</tbody>
</table>
Patients with a raised Hb A₂ and no β-thalassaemia

25 patients had a normal β-globin gene sequence
average values: Hb A₂   MCH   MCV
3.8       28.8    87

• 3 had MCH below 27 pg with normal α-genotype
• Possible causes: mutation in LCR or enhancer sequence

• 21 had a MCH above 27pg: Are these patients normal?
• Possible known causes:
  HIV drug treatment,
  Hyperthyroidism
• Is it the tail end of the range for normal individuals?
UKNEQAS: UK National External Quality Assessment Scheme

- Participants are required to give analytical results and an interpretation

- With increase in technologies:
  - Results of Hb $A_2$ measurement related to methodology used
  - Identified differences in values obtained from different technologies and/or kits
Normal sample: Hb A\textsubscript{2} 2.6%
Beta thal trait sample: Hb A₂ 4.8%
Borderline sample: Hb A$_2$ 3.7%
Performance scoring for Hb A$_2$: Considerations for UKNEQAS

- Use of different normal ranges – variation even within same instrument group
- Use of a universal cut-off
  Instrument bias – impact on borderline values
Measurement of Hb A$_2$

ICSCH recommendations ISLH Oct 2011

• Previous ICSH recommendations written in 1978

• Hb A$_2$ is measured as a percentage of haemoglobin present relative to any other haemoglobin present – not an absolute value

• Therefore analytically important to measure the A$_2$ and any other fractions present – separation, resolution and integration crucial

• In the presence of an Hb A$_2$ variant, it is the total of the normal and abnormal Hb A$_2$ which is significant
ICSH recommendations ISLH Oct 2011

- Fraction separation by
  - Electrophoresis with elution or microcolumn chromatography
  - Quantification by spectrophotometry at 415nm

- HPLC

- Capillary Zone Electrophoresis

- Capillary Isoelectric Focusing

- DNA analysis is required for the characterization of
  - beta thalassaemia mutations
ICSH recommendations ISLH Oct 2011

- Measurement of the Hb A$_2$ alone cannot absolutely confirm or exclude the carrier state as there may be little difference between A$_2$ in normals and some beta thalassaemia carriers.

- Precision levels should be +/- 0.1% of the final answer (SD 0.05%).

- Common beta thalassaemia trait Hb A$_2$ = 4.0 - 6.0%.
- Beta thalassaemia trait overall usually Hb A$_2$ = 3.5 - 7.0%.
- Normal subjects usually Hb A$_2$ = 2.2-3.3%.
Current developments

• Instrument calibration-use of calibrant(s)

• Target value for performance scoring:
  • all methods mean
  • method-specific mean – current target
  • submethod-specific mean

• Development of new Hb $A_2$ reference material
Acknowledgements

For information on beta thalassaemia mutations in Europe:
  Dr John Old
  National Haemoglobinopathy Reference Laboratory, Oxford

For assessment of Hb A₂ in UKNEQAS scheme:
  Barbara Dela Salle, UKNEQAS
  Hannah Batterbee, Royal Hallamshire Hospital / UKNEQAS