The haemoglobinopathies:

Where are mistakes made?

Barbara Wild

Haemoglobinopathy Specialist Consultant

UK NEQAS Haematology

UK NEQAS
Haematology and Transfusion

UKNEQAS Haematology Quality assessment schemes for the Haemoglobinopathies

- Abnormal Haemoglobins
- Newborn sickle screening on dried blood spots
- DNA diagnostics for the Haemoglobinopathies

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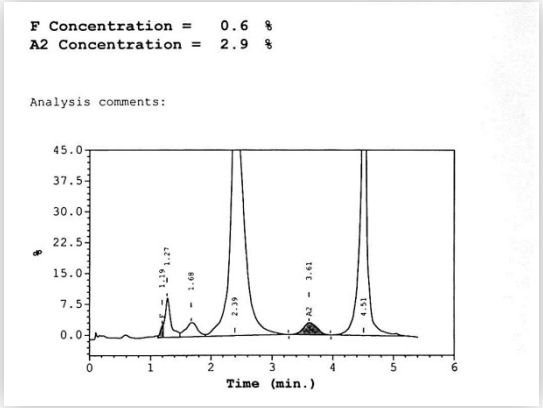
Haemoglobinopathy schemes

Sickle screening

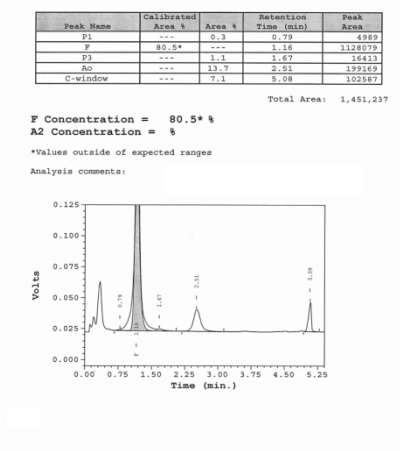
Solubility test

Abnormal haemoglobins+HbA_2/F

Haemoglobin electrophoresis
High Performance Liquid Chromatography
Capillary electrophoresis
Mass spectrometry



Liquid newborn specimens



Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
P1	---	0.3	0.79	4989
F	80.5*	---	1.16	1128079
P3	---	1.1	1.67	16413
Ao	---	13.7	2.51	199169
C-window	---	7.1	5.08	102587

Total Area: 1,451,237

F Concentration = 80.5* %
A2 Concentration = %

*Values outside of expected ranges

Analysis comments:

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Abnormal hb's surveys

Simulate specimens from adult 'patients'

Application: For the detection, presumptive identification and interpretation of haemoglobinopathies

- Utilises blood from NHSBT
- Normal donor blood often manipulated to produce an abnormality
- Creative writing used to produce a case study

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Specimen1701AH1

Case details given:

31 years old female of African origin attending for antenatal booking

Hb = 125 g/L

RBC = 4.26 10^{12}/L

MCV = 86.6 fl

MCH = 29.3 pg

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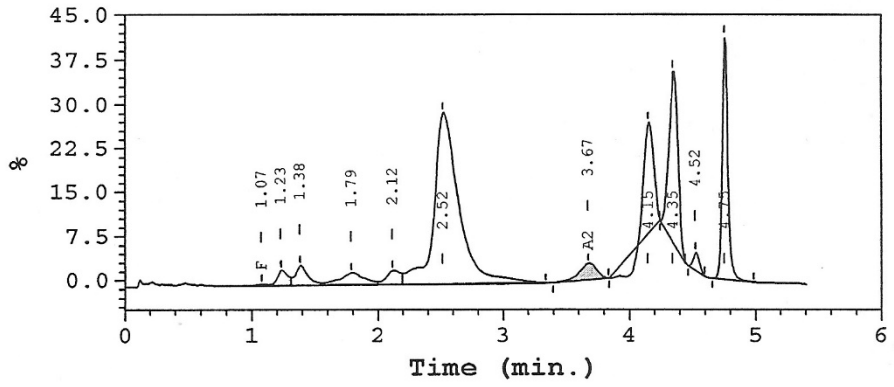
1701AH1: Pre-acceptance testing at UK NEQAS

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	0.2	---	1.07	2467
Unknown	---	1.8	1.23	27333
P2	---	2.5	1.38	39439
P3	---	3.1	1.79	48550
Unknown	---	2.2	2.12	33366
Ao	---	44.9	2.52	694892
A2	2.9	---	3.67	46777
D-window	---	11.6	4.15	179073
S-window	---	15.8	4.35	245231
Unknown	---	1.1	4.52	17540
Unknown	---	13.8	4.75	214430

Total Area: 1,549,096

F Concentration = 0.2 %
A2 Concentration = 2.9 %

Analysis comments:



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Hb G Philadelphia trait α68 (Asn>Lys)

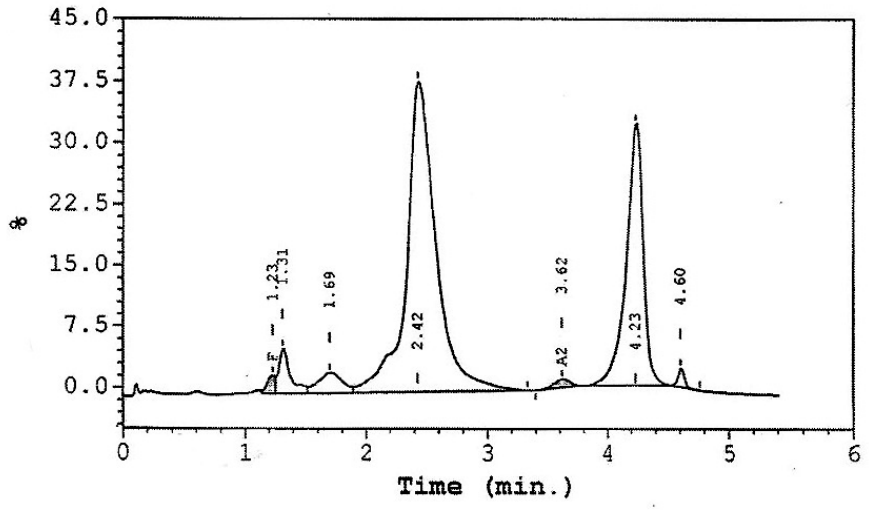
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	1.0	---	1.23	25854
P2	---	3.4	1.31	88450
P3	---	3.2	1.69	83353
Ao	---	60.6	2.42	1560836
A2	1.0*	---	3.62	23802
D-window	---	30.0	4.23	773315
S-window	---	0.8	4.60	20111

Total Area: 2575721

F Concentration = 1.0 %
A2 Concentration = 1.0* %

*Values outside of expected ranges

Analysis comments:



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Hb AS + Hb G Philadelphia

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
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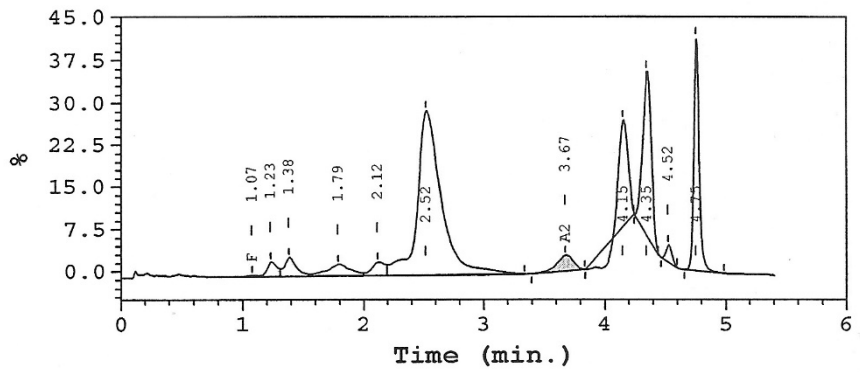

Fractions detected

Hb A	$\alpha_2\beta_2$	44.9 %
Hb A_2	$\alpha_2\delta_2$	2.9 %
Hb'D'	$\alpha^G{}_2\beta_2$	11.6 %
Hb S	$\alpha_2\beta^S{}_2$	15.8 %
Hb A_2G	$\alpha^G{}_2\delta_2$	1.1%
Hb SG	$\alpha^G{}_2\beta^S{}_2$	13.8 %

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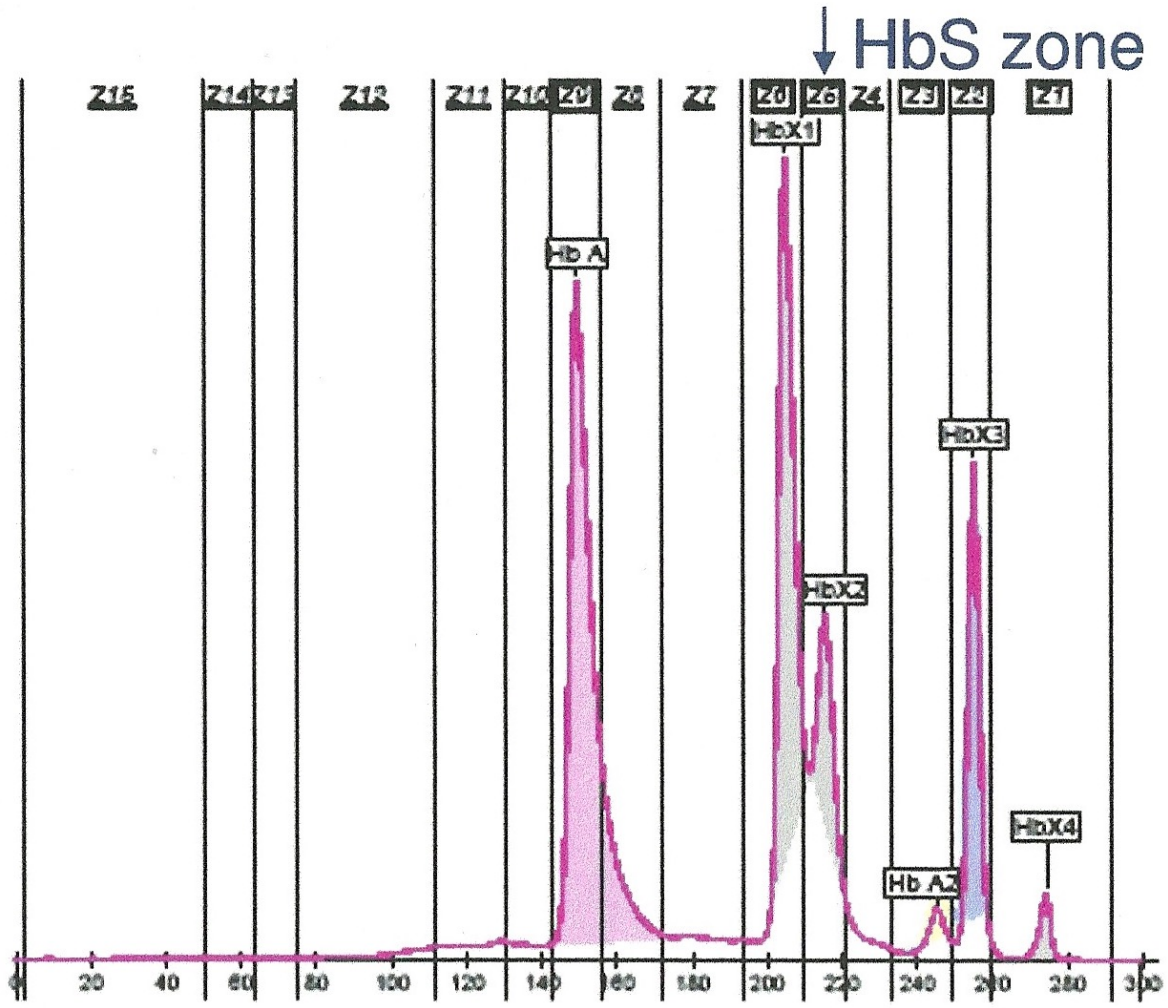
HbAS + Hb G Philadelphia



Fractions detected

Hb A = 45.7 %

Hb G = 27.0 %

Hb S = 6.5 %

Hb A_2 = 2.2 %

Hb SG = 16.6 %

Hb A_2G = 2.0 %

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1701AH1 – Fraction identification

- 319 participants results for fraction identification were used in analysis
- A total of 38 different combinations of hb fractions were reported
- Essential fractions were : Hb A and Hb S
  41participants did not state these present

Other fractions reported:

- A_2, F, F1, D, C, E, Non-specified fractions
- Some participants reported Hb G possible in their interpretation

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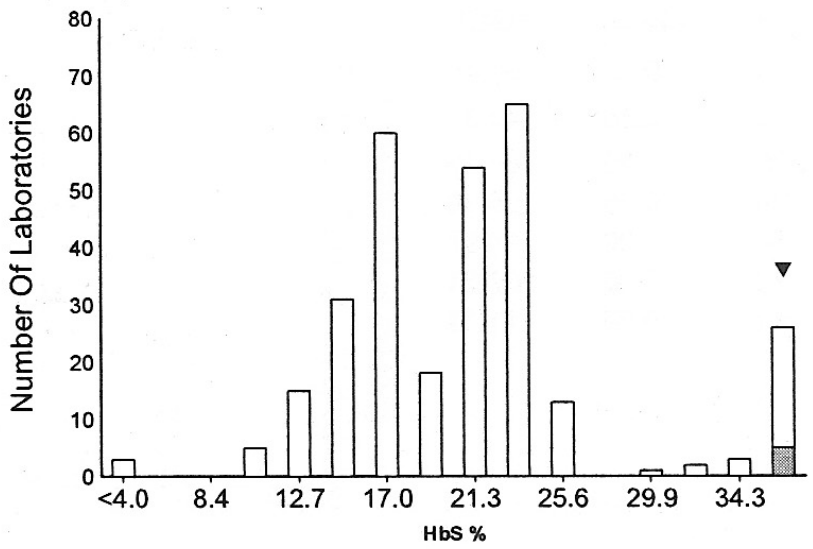
1701AH1 – Fraction identification

Fraction Quantitation

Haemoglobin S (%)

	n	Mean	GCV
All Methods	294	19.2	33.78
Capillary Electrophoresis	52	13.6	21.41
Sebia Capillarys	17	13.9	18.10
Sebia Capillarys 2	25	13.4	23.33
HPLC	237	20.3	26.04
Arkray HA-8180T	18	19.7	5.31
Arkray HA8160	19	36.0	23.79
BioRad D10; Dual Program Kit	23	23.1	8.87
BioRad Variant II; Beta-thal short pro	66	16.3	6.28
BioRad Variant II; Dual program Kit	22	24.0	7.60
Primus Ultra 2	17	20.9	11.99
TOSOH G8	47	20.7	6.43



Reported range for Hb S = 0.0–45.10%

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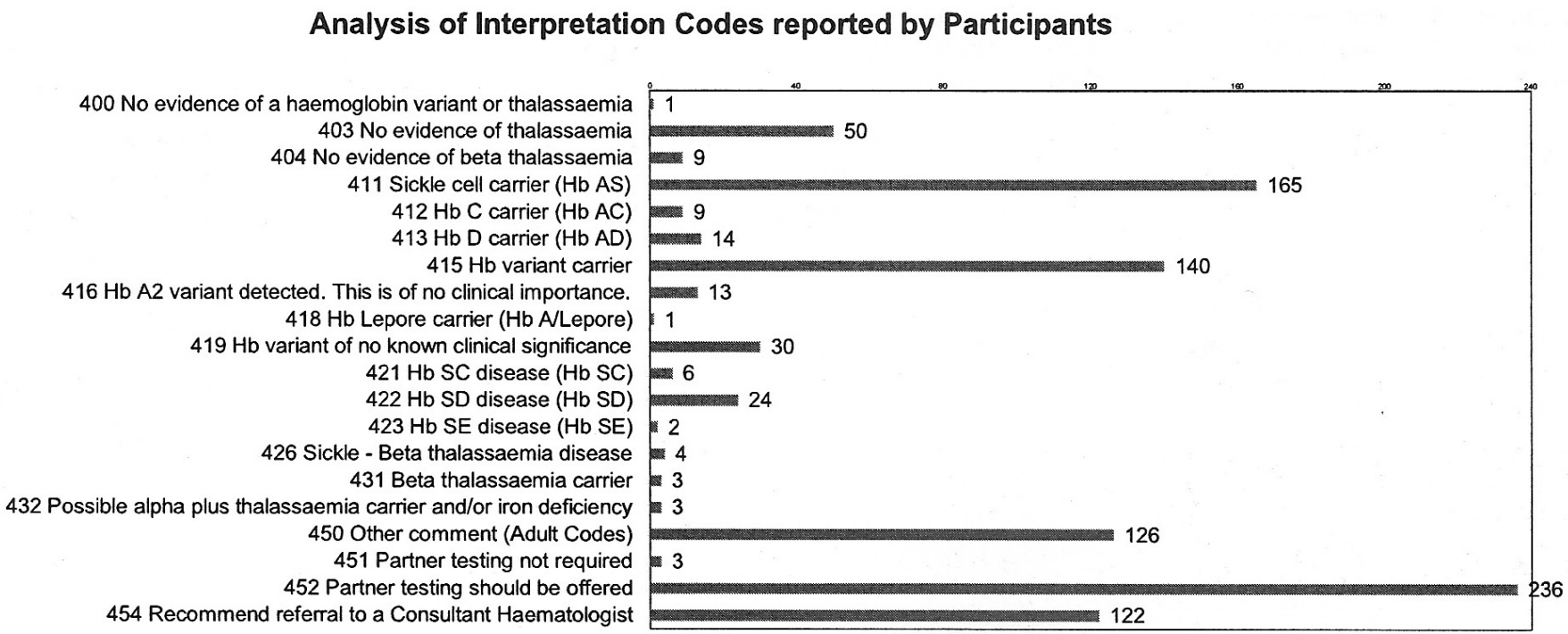
1701AH1 – Interpretive comments



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1701AH1 – Interpretive comments

335 participants results for interpretive comments were used for the UK NEQAS report

- 164 participants: Sickle cell trait
  Represents 49% of all returns
- 148 of the 164: Partner testing recommended
- 5 of the 148: Commented there was a second variant present
  Represents 3% of all returns

Of the total returns, 70% stated partner testing should be undertaken

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Where were the ‘mistakes’?

Using different analysers:

- Variation in separations and proposed identity
- Variation in quantitation of haemoglobin components
- Variation in Hb S quantitation confusing re the diagnosis of sickle cell trait

Where were the ‘mistakes’?

Initial identity of donor’s haemoglobinopathy:
Inaccurate – ? Only tested by solubility

Quantitation of Hb S:
Inaccurate and specimen unsuitable for scoring