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A Most Interesting Haemoglobinopathy

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Case 5
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- A most interesting EQA exercise…
- “Split Hb A_2” (alpha or delta chain variant)
- UK NEQAS Haematology – Abnormal Haemoglobins Programme
- Specimen 2303AH1 (dispatched in June 2023)

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- A 24-year-old woman of African ethnicity undergoing antenatal screening.
- The patient had normal red cell indices
  - RBC (1012/L) 4.19
  - Hb (g/L) 115
  - MCV (fL) 83.8
  - MCH (pg) 27.4
- Haemoglobinopathy screening showed the presence of a Hb A_2 variant.

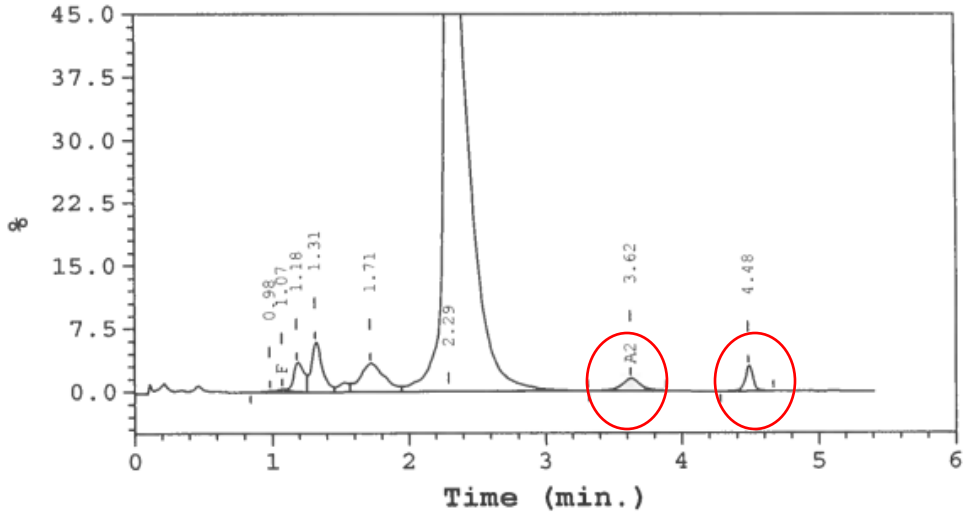
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.98	1481
F	0.3	---	1.07	5766
Unknown	---	2.0	1.18	46547
P2	---	3.5	1.31	80482
P3	---	4.4	1.71	99368
Ao	---	86.9	2.29	1975566
A2	1.5*	---	3.62	34804
S-window	---	1.3	4.48	29079

Total Area: 2,273,093

F Concentration = 0.3 %
A2 Concentration = 1.5* %

*Values outside of expected ranges

Analysis comments:



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Quick poll:

How does your laboratory report *patients* with a split Hb A_2 peak?

(Please answer honestly)

1. We only report the HbA_2 detected in the HbA_2 zone/window
2. We add the two fractions together and report “total” HbA_2
3. I don’t know!

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- So, how did our participants do?
- We didn’t tell you at the time because…
- 2303AH1 was withdrawn from scoring

Why?

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- 2303AH1 was withdrawn from performance assessment for Hb A_2 %.
- This means participants did not get a “DI” (Deviation Index) for this specimen.

  Reminder:

  – *DI (equivalent to a statistical z-score) tells you how far away your result was from the target.*

  – *The DI is used to calculate the analytical performance score*
- This is because some participants only reported the fraction identified as Hb A_2 by their analyser; others added the two Hb A_2 fractions together and reported the “total” Hb A_2

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- When asked to make an assessment of the Hb A_2 %:
  - 43% of participants reported it as ‘low’
  - 54% of participants reported it as ‘normal’
- The range of results returned for Hb A_2: 0.3% - 3.9%
- There was no difference in approach between HPLC and CE users

- There was a bimodal distribution of results
- The all methods Geometric Coefficient of Variation (GCV) was 53.04%

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Haematology and Transfusion

Abnormal Haemoglobins Scheme | **Laboratory:**

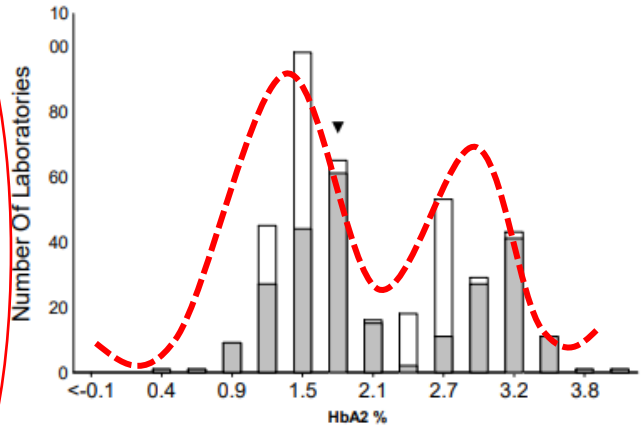
Distribution: 2303AH **Date: 05 Jun 2023** | **Page 3 of 10**

Specimen : 2303AH1

Fraction Quantitation
Haemoglobin A2 (%)

Bimodal distribution

	n	Mean	GCV
All Methods	391	1.8	53.04
Capillary Electrophoresis	135	1.7	45.87
Sebia Capillarys	18	2.0	43.05
Sebia Capillarys 2	41	1.7	45.32
Sebia Capillarys 3	47	1.8	44.88
Sebia Minicap	25	1.5	30.63
HPLC	252	1.9	55.90
Arkray HA-8180T	22	1.7	12.91
BioRad D10; Dual Program Kit	13	1.9	24.16
BioRad Variant II; Beta-thal short pro	82	2.3	41.69
BioRad Variant II; Dual program Kit	33	1.5	50.31
Hb9210 Resolution	18	1.8	54.10
TOSOH G11	34	1.8	62.33
TOSOH G8	32	1.7	94.40



Your registered method is:
HPLC BioRad Variant II;
Beta-thal short program

Your Result :	1.6
DI :	-2.39
Uncertainty of Method Mean :0.12	
Perf Score :	39.3
Reported Range (Overall)	
Minimum	0.30
Maximum	3.90
Assessment vs your ref range	
You reported:	Low
Overall Assessment (%)	
Low	43.2
Normal	54.0
High	0.8
Uncertain	2.1

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Haematology and Transfusion

Abnormal Haemoglobins Scheme | **Laboratory:**

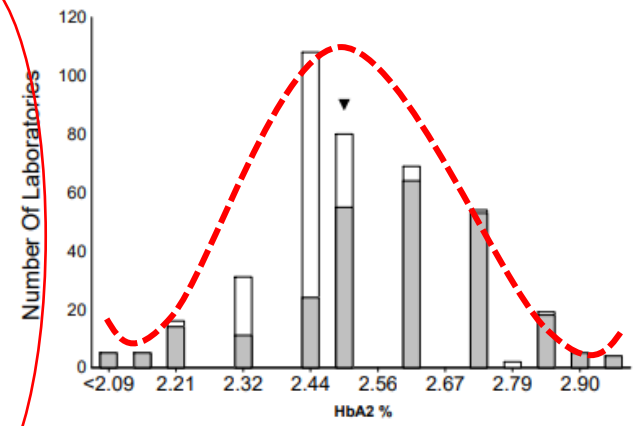
Distribution: 2303AH Date: 05 Jun 2023 |

Specimen : 2303AH2

Fraction Quantitation
Haemoglobin A2 (%)

	n	Mean	GCV
All Methods	398	2.5	6.89
Capillary Electrophoresis	136	2.4	2.73
Sebia Capillarys	18	2.4	3.34
Sebia Capillarys 2	41	2.4	2.16
Sebia Capillarys 3	47	2.4	2.50
Sebia Minicap	26	2.4	2.53
HPLC	258	2.6	7.24
Arkray HA-8180T	25	2.6	5.10
BioRad D10; Dual Program Kit	13	2.7	5.29
BioRad Variant II; Beta-thal short pro	83	2.6	3.73
BioRad Variant II; Dual program Kit	33	2.5	7.27
Hb9210 Resolution	19	2.6	7.85
TOSOH G11	34	2.5	5.61
TOSOH G8	32	2.4	8.24

Normal distribution



Your registered method is:
HPLC BioRad Variant II;
Beta-thal short program

Your Result :	2.5
DI :	-0.01
Uncertainty of Method Mean :0.08	
Perf Score :	39.3
Reported Range (Overall)	
Minimum	1.50
Maximum	3.90
Assessment vs your ref range	
You reported:	Normal
Overall Assessment (%)	
Low	1.0
Normal	98.7
High	0.3
Uncertain	

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Taking a closer look at the results returned…

- 391 participants returned results for Hb A_2 quantification
  - 153/391 (39%) were UK labs
  - 238/391 (61%) were non-UK labs

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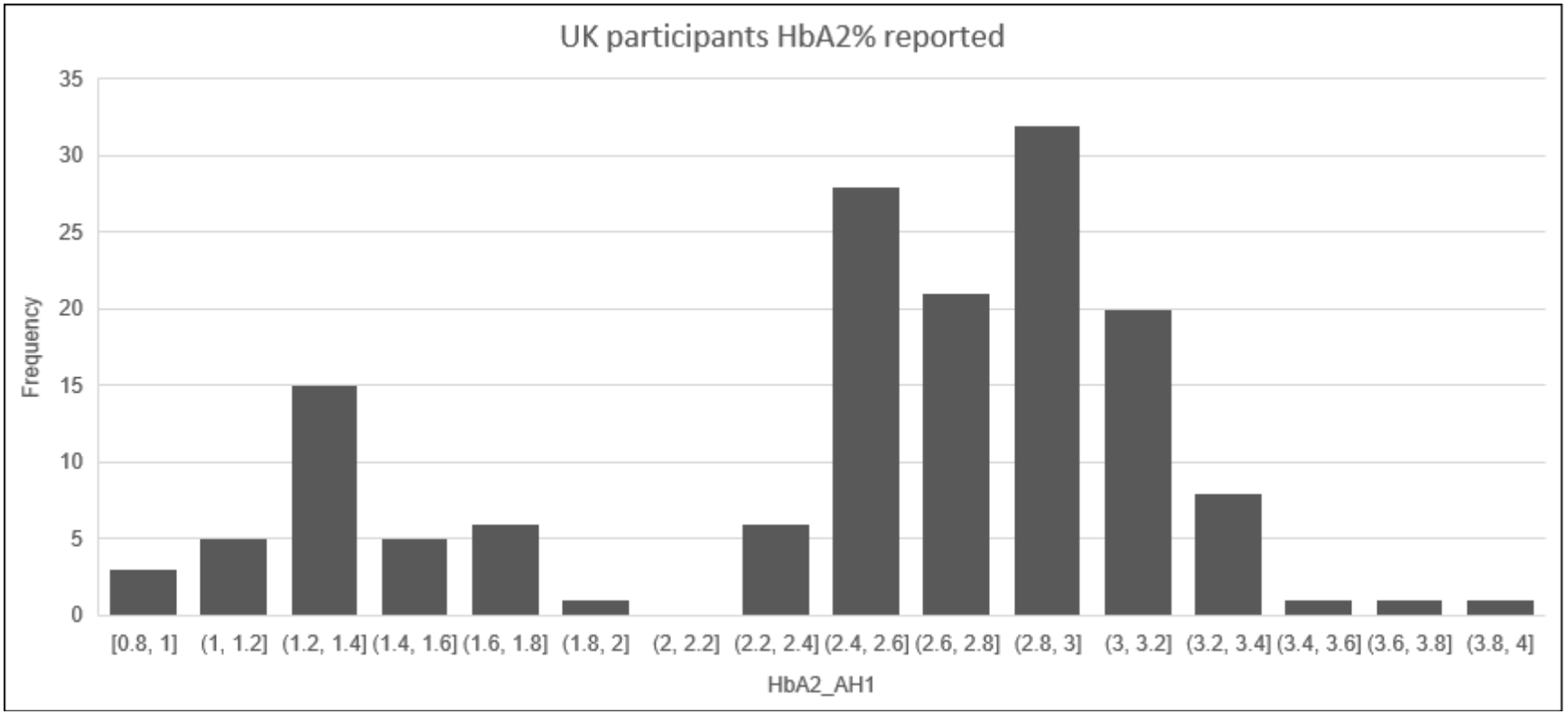

NHS (English Trusts)	130
NHS (Non-English Trusts)	16
Other	5
UK private sector	2
Grand Total	**153**

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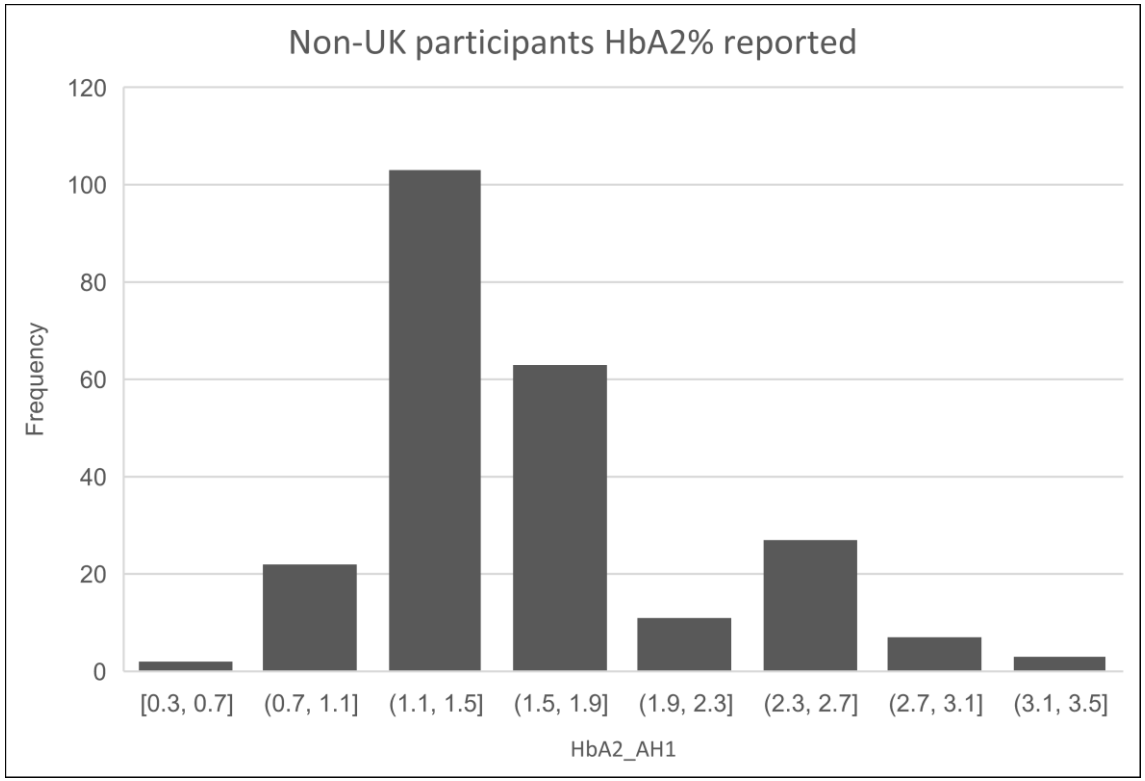

Europe	152
Outside Europe	86
Grand Total	**238**

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Common questions we get:

- *“Why don’t I have a DI for this specimen?”*
- *"We don’t want to get an adverse score!”*
- *“We don’t want our analytical performance score to increase!”*
- *“How should we report these results?”*
- *“Please tell us what to do…”*

The short answer is:

No. We can't tell you what to do ☹

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Here is a friendly reminder:

- You should treat EQA specimens *exactly the same* as patient samples
- Follow your existing laboratory protocols

Case 5

- We can't tell you what to do…
- But we can point you towards existing recommendations and guidelines… ☺

British Society for Haematology:

- Significant haemoglobinopathies: A guideline for screening and diagnosis (b-s-h.org.uk)

International Society for Laboratory Haematology:

- ICSH recommendations for assessing automated high-performance liquid chromatography and capillary electrophoresis equipment for the quantitation of HbA2 - Stephens - 2015 - International Journal of Laboratory Hematology - Wiley Online Library

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- What the guidelines say:

ously [3], it is important to both detect and quantitate any HbA_2 variant that is present (due to either an α- or δ-globin chain mutation) and include it in the total HbA_2 reported. If this is not performed, people who also carry β-thalassemia may be missed.

- ISLH

Conclusions

- The presence of an Hb A_2 variant reduces the Hb A_2 concentration detected by the analyser and could result in a beta thalassaemia carrier being missed.
- There is still significant variation in the way laboratories are reporting these types of cases.
- Performance assessment from an EQA perspective is difficult but cases like this have important educational value

Any questions: email me/us at haem@ukneqas.org.uk

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That’s it