The haemoglobinopathies: Learning from EQA

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Haematology and Transfusion

UKNEQAS Haematology Quality assessment schemes for the Haemoglobinopathies

- Sickle cell screening
- Abnormal Haemoglobins HbA_2/F
- Liquid Newborn specimens

- Newborn sickle screening on dried blood spots

- DNA diagnostics for the Haemoglobinopathies

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Haemoglobinopathy schemes

- Sickle screening

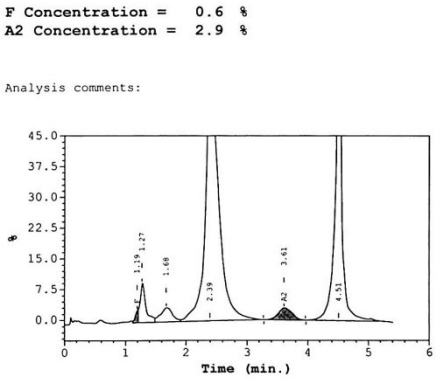
  Solubility test

Specimens:
Whole blood

- Abnormal haemoglobins + HbA_2/F

Haemoglobin electrophoresis
High Performance Liquid Chromatography
Capillary electrophoresis

Mass spectrometry



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Liquid newborn specimens

Peak Name	Calibrated Area %	Area %	Retention Time (min)
F	89.3*	---	1.18
P3	---	0.9	1.73
Ao	---	15.4	2.58

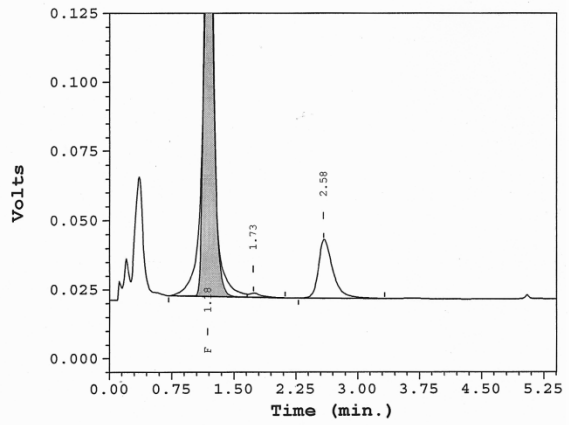
Total Area:

F Concentration = 89.3* %
A2 Concentration = %

*Values outside of expected ranges

Analysis comments:

F+A



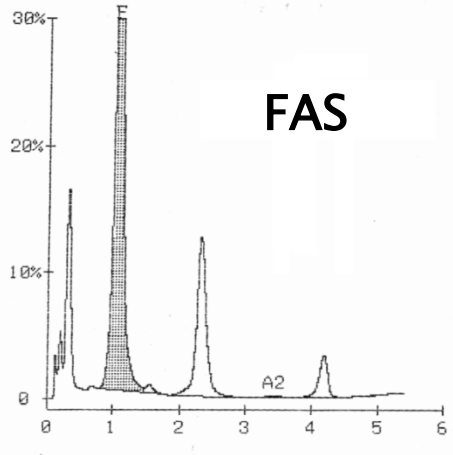
*** Beta Thal Short 90650-A ****
DATE:20/11/13 TIME:17:22:44

TECH ID# 2
VIAL# 5
SAMPLE ID# 00000000000000000000

ANALYTE ID	%	TIME	AREA
F	75.3	1.08	937447
P3	0.9	1.54	11249
Ao	21.4	2.32	262899
A2	0.3	3.43	3352
S-WINDOW	5.1	4.18	62284
TOTAL AREA			1277231

F 75.3% A2 0.3%

FAS



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

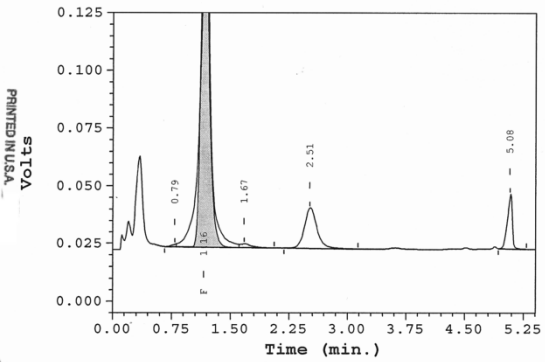
Total Area: 1,451,237

F Concentration = 80.5* %
A2 Concentration = %

*Values outside of expected ranges

Analysis comments:

FAC



Suitable for HPLC, CE, IEF

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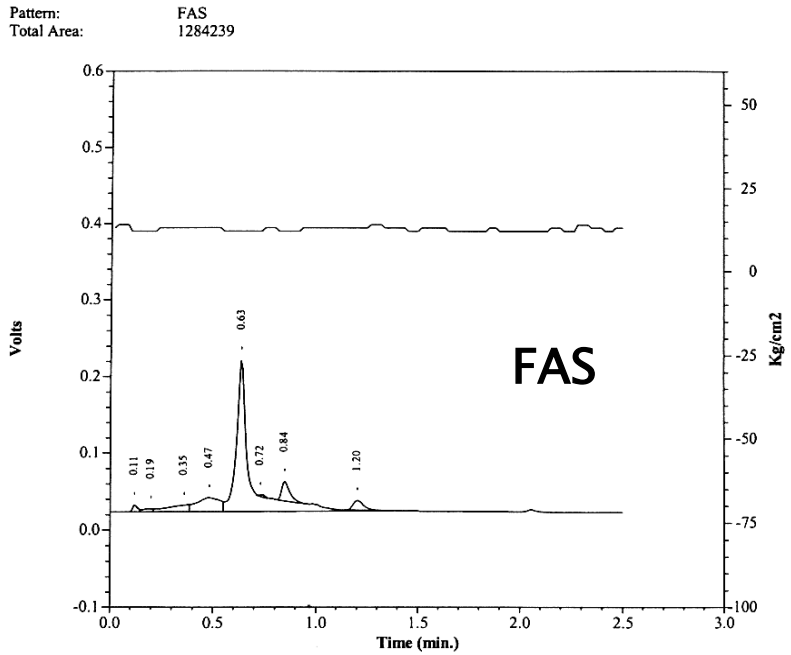
Haemoglobinopathy schemes

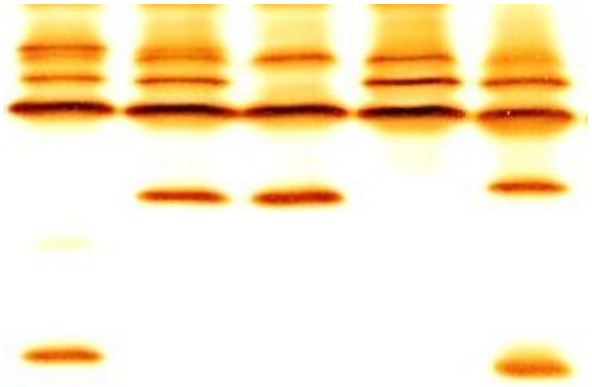
Newborn screening for sickle cell disease

High Performance Liquid Chromatography



Specimens:
Dried blood spots

Isoelectric focusing



FAC FAS FS FA AFDC

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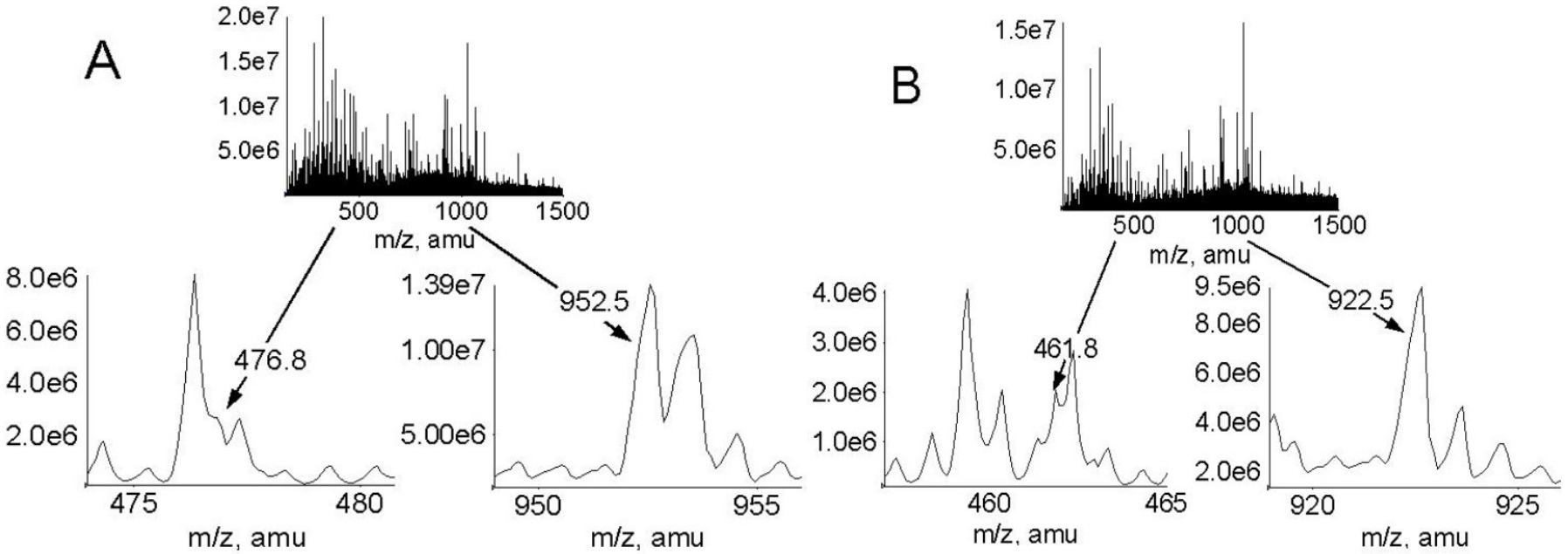
Haemoglobinopathy schemes

Newborn screening for sickle cell disease:
Mass Spectrometry



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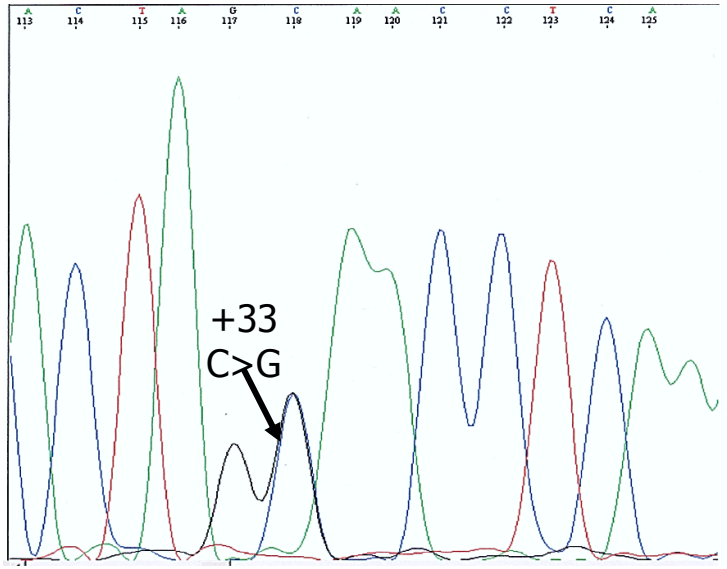
Haemoglobinopathy schemes

- DNA diagnostics

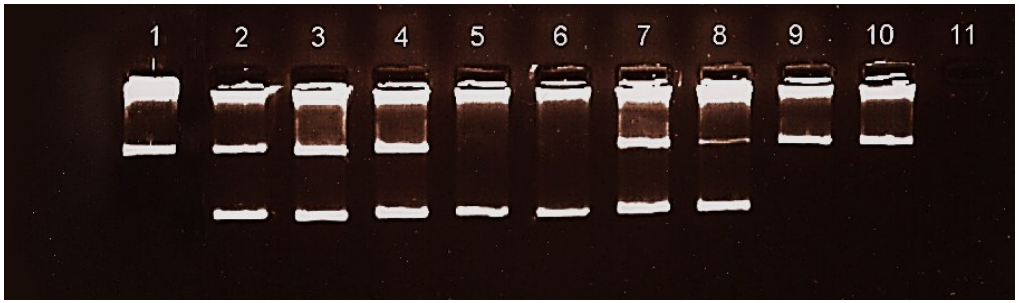
Specimens:
Extracted DNA in buffer

Suitable for all DNA diagnostic techniques

DNA fluorescent sequencing



Amplification Refraction Mutation System



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What have the Haemoglobinopathy schemes highlighted?

- Are the analyses undertaken both appropriate and comprehensive
- Can they result in interpretations given
- Does the report answer the question
- Are recommendations given both appropriate
- Are coded comments too limiting

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Liquid newborn scheme

Purpose:
To emulate the scenario of clinician-led testing on infants potentially at-risk for a major haemoglobinopathy

- Analyses are undertaken on whole blood
- Received as a pathology request in a haematology department
- Specimen accompanied by family history
- Results should be available within a day or so

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Liquid newborn specimens
Case 1603LN2

Details accompanying specimen:

The specimen is from a one day old Nigerian female infant. Her FBC is normal. Her mother is a known sickle cell carrier, the father is said to be a carrier for Hb C, but no written report is available.

1603LN2: Fraction identification

Results confirmed the infant to be a carrier for Hb S – Fraction identification: Hb F+A+S

Methods used

- All Participants: 17/38 laboratories who returned results had only undertaken one analytical method
- UK participants: 14/31 undertook single method only*

** although one stated confirmatory testing required*

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1603LN2: Fraction identification

Other references to a confirmatory method:

- Sickle solubility not performed due to low level of Hb S
- Sickling test positive
- Hb S level approx 6%

1603LN2: Interpretive comments

- Appropriate interpretation of ‘consistent with sickle cell carrier’ used

However

- 7/38 participants that testing should be repeated at 6 months of age
- 1participant stated that testing should be repeated at one year of age

Does this imply that detection of sickle haemoglobin in newborns is an inconclusive procedure??

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Liquid newborn specimens: Case 1603LN1

Details accompanying specimen:

The specimen is from a one day old Asian male infant. His FBC is normal. His parents are both carriers for beta thalassaemia.

1603LN1: Results

Results showed the presence of Hbs F and A, consistent with a normal result for a newborn.

Methodology used

- All participants: 8/38 laboratories undertook two methods of analysis

- UK participants: 7/31 laboratories undertook two methods of analysis

Confirmation of presence of Hb A would be a wise precaution in this case, because of the high risk of beta thalassaemia major

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1603LN1: Interpretive comments

- All participants gave comment code 700, ie:

'No common haemoglobin variant detected: beta-thalassaemia trait cannot be excluded.'

- This is the nearest match *if* using the coded comments list.

- Free text is always an option – surely preferable in this case???

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1603LN1: Interpretive comments

Other comments included:

- Suggest repeat at over 2 years of age (*n=1*)
- Suggest repeat at over 1 year of age (*n=2*)
- Suggest repeat at an ***appropriate*** age (*n=1*)
- Repeat in 6 months (*n=3*)
- Valid if not transfused (*n=4*)

- No evidence of beta thalassaemia major *(n=2)*

Considerations

- This type of testing is not population screening
- The interpretive comment used – although the nearest ‘match’ is not helpful to the clinician
- Should we encourage the use of ‘free text’

Liquid newborn scheme

What about performance assessment?

Within the DNA diagnostics scheme:

“Interpretation and recommendations must be accurate, appropriate and complete”

Suggesting repeat testing for a sickle carrier infant is not appropriate

Excluding the presence of a hb variant when beta thalassaemia major is being queried is not answering the question

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Newborn sickle screening

General overview of results:

- Interpretation of results concise and accurate
- Repeat testing at six months sometimes recommended
- Secondary testing methods omitted in some cases

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Newborn sickle screening

Performance assessment in general

- Repeat testing *in general* is not National Screening Policy for carriers

–should this recommendation be penalised

- In contrast to the DNA scheme, the 'protein' techniques give a presumptive identification
- If good practice guidelines state more than one method is necessary

–should the lack of such be penalised

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Abnormal haemoglobin scheme

1603AH3:

- 29 year old Nigerian female undergoing antenatal haemoglobinopathy screening
- Analytical results: Hb C carrier

Abnormal haemoglobins scheme

- From a total of 309 participants

60 did not report the essential haemoglobin fractions for this case

Hb's A + C

26 participants reported the presence of a '*non-specific fraction*'

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Abnormal haemoglobins scheme

What is expected in this case:

- Report stating Hb C carrier

(and the need to undertake partner testing)

- Issuing a ‘safe report’ is more suitable than an inconclusive result

- Should we remove the ‘non-specified fraction’ and score on different levels of testing

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Lessons learned from the DNA diagnostics sheme

Performance is assessed in different categories

- Mutation analysis
- Correct interpretation of the analyses in conjunction with the FBC and case details
- Follow up recommendations
- Nomenclature

All of above compared to a Model Answer

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Challenges

Changes in asssessment of the ‘protein’ based schemes could include

- Creation of a tier-based system where laboratories can ‘refer’ or issue a part report
- Derivation of a model answer
- Bespoke scoring criteria for each case scenario
- Differentiation between screening and diagnostic cases and consideration of generic comments

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