The haemoglobinopathies: Learning from EQA

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UKNEQAS Haematology Quality assessment schemes for the Haemoglobinopathies

- Sickle cell screening
- Abnormal Haemoglobins HbA₂/F
- Liquid Newborn specimens

- Newborn sickle screening on dried blood spots
- DNA diagnostics for the Haemoglobinopathies
Haemoglobinopathy schemes

- Sickle screening
  - Solubility test

- Abnormal haemoglobins $+\text{HbA}_2/F$
  - Haemoglobin electrophoresis
  - High Performance Liquid Chromatography
  - Capillary electrophoresis
  - Mass spectrometry

Specimens: Whole blood
Suitable for HPLC, CE, IEF
Haemoglobinopathy schemes

Newborn screening for sickle cell disease

High Performance Liquid Chromatography

Specimens:
Dried blood spots

Isoelectric focusing

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Newborn screening for sickle cell disease: Mass Spectrometry

![Graph A](image)

![Graph B](image)

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- DNA diagnostics
  
  Suitable for all DNA diagnostic techniques

Specimens:
Extracted DNA in buffer

Amplification Refraction Mutation System

DNA fluorescent sequencing

[Image of gel electrophoresis with bands labeled 1 to 11, and DNA sequence trace indicating +33 C>G mutation]
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
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
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.
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
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
- 1 participant 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??
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.
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.
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???
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
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
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
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’
Abnormal haemoglobinins 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
Lessons learned from the DNA diagnostics scheme

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
Challenges

Changes in assessment 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