UK NEQAS (H)

Perspective on performance:
The Haemoglobinopathies

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Haemoglobinopathies

Perspective on Performance:
Performance assessment process

DNA diagnostics for Haemoglobinopathies scheme
Outcomes of shadow scoring exercise

Abnormal Haemoglobins $A_2/F/S$ scheme
Plan for performance assessment of interpretive comments
UKNEQAS(H)
Quality assessment schemes for the Haemoglobininopathies

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

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

- Sickle screening
  - Solubility test

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

Specimens: Whole blood

Liquid newborn samples
Haemoglobinopathy schemes

- Newborn screening for sickle cell disease

High Performance Liquid Chromatography

Specimens: Dried blood spots

Isoelectric focusing

Mass Spectrometry
Haemoglobinopathy schemes

△ DNA diagnostics

Suitable for all DNA diagnostic techniques

Specimens: Extracted DNA in buffer

Amplification Refraction Mutation System

DNA fluorescent sequencing

+33 C>G
DNA scheme: Plan

- Develop schedule for regular surveys
- Develop Performance Assessment
- Apply for accredited status of scheme
Scheme commenced in 2002 as a pilot scheme

Purpose:

› To assess the quality of DNA analyses for the haemoglobinopathies within the UK

Participants:

› The 3 Prenatal Diagnosis laboratories
› Any other UK laboratory undertaking α/β genotyping

Surveys:

› 12 surveys over 10 year period

Outcome:

› Summary reports, no scoring undertaken
Current process

Schedule developed
3 surveys per with 2 specimens per year, commenced 2011

Specimens issued with:
Age/gender
Full blood count data
Haemoglobinopathy screening data
Reason for referral
## Current participants

<table>
<thead>
<tr>
<th>Country</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>1</td>
</tr>
<tr>
<td>Austria</td>
<td>2</td>
</tr>
<tr>
<td>Belgium</td>
<td>3</td>
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<tr>
<td>Cyprus</td>
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<tr>
<td>France</td>
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<tr>
<td>Germany</td>
<td>2</td>
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<tr>
<td>Greece</td>
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<tr>
<td>Netherlands</td>
<td>2</td>
</tr>
<tr>
<td>Israel</td>
<td>2</td>
</tr>
<tr>
<td>Ireland</td>
<td>2</td>
</tr>
<tr>
<td>Portugal</td>
<td>3</td>
</tr>
<tr>
<td>Poland</td>
<td>1</td>
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<tr>
<td>Spain</td>
<td>1</td>
</tr>
<tr>
<td>Sweden</td>
<td>2</td>
</tr>
<tr>
<td>Switzerland</td>
<td>4</td>
</tr>
<tr>
<td>UK</td>
<td>9</td>
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</tbody>
</table>

Total = 43 participants

16 different countries
Current process

**Start**
- Specimens and instructions dispatched
- Participants given 4 weeks to complete

**Data Analysis**
- Summary report written → participants
- *Results shadow scored*

**Outcomes**
- Results and recommendations →
- Expert advisors, SSAG + NQAAP
Shadow scoring

> Scoring requires internal and external expert

> Model answer agreed by experts

> Assessment undertaken at UKNEQAS(H)

> Independent scoring by external assessor

> Meeting to finalise participants’ scores
## Tariff of penalties

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Penalty</th>
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<tr>
<td>Non participation</td>
<td>50</td>
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<tr>
<td>Incorrect analytical results: $\alpha$ genotype</td>
<td>50</td>
</tr>
<tr>
<td>Incorrect analytical results: $\beta$ genotype</td>
<td>50</td>
</tr>
<tr>
<td>Incorrect annotation: $\alpha$ genotype</td>
<td>35</td>
</tr>
<tr>
<td>Incorrect annotation: $\beta$ genotype</td>
<td>35</td>
</tr>
<tr>
<td>Incorrect interpretation re case details</td>
<td>50</td>
</tr>
<tr>
<td>Incorrect annotation of interpretation</td>
<td>35</td>
</tr>
<tr>
<td>Inadequate/absent/incorrect recommendations</td>
<td>50</td>
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<tr>
<td>HGVS nomenclature incorrect/not used</td>
<td>35</td>
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</table>
## Cases shadow scored

<table>
<thead>
<tr>
<th>Date</th>
<th>Genotypes : Specimen 1</th>
<th>Genotypes : Specimen 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 2012</td>
<td>$-\alpha_{SEA}/-\alpha_{3.7} : \beta^A/\beta^E$</td>
<td>$-\alpha_{4.2} /\alpha\alpha : \beta^A/\beta^{IVS1-5} (G&gt;C)$</td>
</tr>
<tr>
<td>November 2012</td>
<td>$\alpha\alpha /\alpha\alpha : \beta^A/\beta^{Fr , 41-42(-TCTT)}$</td>
<td>$-\alpha_{3.7}/-\alpha_{3.7} : \beta^A/\beta^S$</td>
</tr>
<tr>
<td>February 2013</td>
<td>$-\alpha_{3.7}/\alpha\alpha : \beta^A/\beta^{{IVS , II , 654(C&gt;T)}}$</td>
<td>$\alpha\alpha /\alpha\alpha : \beta^A/\beta^A$</td>
</tr>
<tr>
<td>July 2013</td>
<td>$\alpha\alpha /\alpha\alpha : \beta^A/\beta^{Cd8/9(+G)}$</td>
<td>$-\alpha_{3.7}/\alpha\alpha : \beta^A/\beta^{-88(C&gt;T)}$</td>
</tr>
</tbody>
</table>
Sample 1202DN1 was from a 1.5 year old female of Vietnamese origin. Referred for elucidation of FBC results:

FBC:  
Hb: 98 g/L  
RBC: 6.43 x 10^{12}/L  
MCV: 52 fl  
MCH: 17 pg

Haemoglobinopathy screen:  
Hb A + fraction eluting in Hb A₂ window  
Hb A₂=13.7%; Hb F=2.0%
## Mutation analysis:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Alpha genotype:</strong></td>
<td>$\alpha^{3.7}$/SEA</td>
</tr>
<tr>
<td><strong>Beta genotype:</strong></td>
<td>$\beta^A/\beta^E$</td>
</tr>
</tbody>
</table>

## Interpretation using case details:

- HbH disease plus Hb E trait (carrier)

## Recommendations on report:

- Child should be referred for follow-up
- Parental testing recommended

## HGVS nomenclature

- HBB:c.79G>A
<table>
<thead>
<tr>
<th>RESULTS</th>
<th>1202DN1</th>
<th>1202 DN2</th>
<th>1301 DN1</th>
<th>1301DN2</th>
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<tbody>
<tr>
<td>No participants</td>
<td>35</td>
<td>35</td>
<td>41</td>
<td>41</td>
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<tr>
<td>Incorrect analysis: $\alpha$ genotype</td>
<td>$-\alpha^{3.7}/-\alpha^{\text{SEA}}$ 4</td>
<td>$-\alpha^{4.2}/\alpha\alpha$ 4</td>
<td>$-\alpha^{3.7}/\alpha\alpha$ 2</td>
<td>$\alpha\alpha/\alpha\alpha$ 2</td>
</tr>
<tr>
<td>Incorrect analysis: $\beta$ genotype</td>
<td>$\beta^A/\beta^{Cd26(G&gt;A)}$ 0</td>
<td>$\beta^A/\beta^{IVS1,5(G&gt;C)}$ 1</td>
<td>$\beta^A/\beta^{IVSII 654(C&gt;T)}$ 4</td>
<td>$\beta\beta/\beta\beta$ 1</td>
</tr>
<tr>
<td>Incorrect annotation: $\alpha$ genotype</td>
<td>6</td>
<td>2</td>
<td>12</td>
<td>1</td>
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<tr>
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<td>3</td>
<td>3</td>
<td>10</td>
<td>1</td>
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<tr>
<td>Incorrect interpretation</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Incorrect annotation of interpret</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Inadequate/absent/incorrect recommendations</td>
<td>21</td>
<td>12</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>HGVS nomenclature incorrect/absent</td>
<td>9</td>
<td>5</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Labs with ZERO penalties</td>
<td>2</td>
<td>6</td>
<td>3</td>
<td>6</td>
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</table>
## Participation

<table>
<thead>
<tr>
<th>Survey</th>
<th>Total number of participants in scheme</th>
<th>Number of Non-participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1202DN</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>1203DN</td>
<td>37</td>
<td>3</td>
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<tr>
<td>1301DN</td>
<td>41</td>
<td>6</td>
</tr>
<tr>
<td>1302DN</td>
<td>43</td>
<td>3</td>
</tr>
</tbody>
</table>
# Ranges of penalties accrued per sample

<table>
<thead>
<tr>
<th></th>
<th>1202 DN1</th>
<th>1202 DN2</th>
<th>1203 DN1</th>
<th>1203 DN2</th>
<th>1301 DN1</th>
<th>1301 DN2</th>
<th>1302 DN1</th>
<th>1302 DN2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranges of points given</td>
<td>0–190</td>
<td>0–190</td>
<td>0–255</td>
<td>0–240</td>
<td>0–220</td>
<td>0–135</td>
<td>0–255</td>
<td>0–255</td>
</tr>
<tr>
<td>% labs with no points</td>
<td>9</td>
<td>16</td>
<td>6</td>
<td>6</td>
<td>9</td>
<td>18</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
Scoring – outcomes

- Labs are given a score
  0 = No penalties

- Persistent unsatisfactory performance
  = 2 or more errors in 3 surveys
  (usual score accumulated = 100 or more)

Accredited Scheme: PUP referred to a professional overseeing organisation
Outcomes of shadow scoring project

- Report of shadow scoring for participants

- Guidelines / user reference compiled
  - What’s required in the report (already provided)
  - Example of model answer
  - Reference websites for guidance
    - Globin gene server
    - ITHANET
    - HGVS
  - Examples of ‘ideal reports’

Meeting for participants to discuss these

November 2014
Incorrect mutation analysis discussed with participant as soon as survey closes

Model answer issued within a week of survey closure

International experts available for discussion on inconsistencies or out of consensus results
Interestingly......

- Annotation can be addressed
- Is interpretation not a usual process?
- Are recommendations not a usual process?
- How do the latter work for different countries’ culture, economy, and healthcare systems?
Finally

Understanding that from non-UK labs this approach could be for NEQAS reports only

Shadow scoring exercise – accepted commences November 2014
Performance assessment of the Abnormal Haemoglobins HbA₂/F Scheme
Performance assessment: Abnormal Hbs, A₂/F scheme

Extension of performance assessment

- Fraction identification
- Interpretive comments

Project to commence 2015
Performance assessment: Abnormal Hbs, A$_2$/F scheme

The process

Likely that the process will be similar to that of the DNA diagnostics

The aim is to achieve:
Performance assessment of the whole analytical, analytical and reporting outcomes
Performance assessment: Abnormal Hbs, A₂/F scheme

There are significant differences to be considered: each individual laboratory’s level of operation, – how they define their role and purpose

- Primary screening, then refer
- Presumptive identification of common variants, then refer
- Comprehensive diagnostic service
- Referral service
Performance assessment: Abnormal Hbs, A$_2$/F scheme

UKNEQAS(H) needs

- more information instrumentation and techniques
- diagnostic protocols

in order to ‘categorise’ laboratories
Performance assessment: Abnormal Hbs, A₂/F scheme

- Consideration of the following

  Modification of the results proforma to encompass all categories of operation

  A good example: Has the ‘Non-specific fraction’ outlived itself

  The level of operation will obviously affect interpretive comments made by participants
Performance assessment: Abnormal Hbs, A₂/F scheme

- Participation – being purist about it.....

- Reasons for repeated non–participation
  - Incomplete participation
  - Failure to request repeat samples
  - Joint participation=one report
Performance assessment: Abnormal Hbs, A₂/F scheme

- Where to start?

- Questionnaire to participants—early next year

- Create participant ‘Groups’

- Modify: results proformas
  - create model answers
  - create new penalty tariff
  - adjust IT accordingly
Performance assessment

Acknowledgements

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