Learning from EQA: ‘Split’ Hb A₂ Peak

Nikki Emodi, Duljeet Chohan, Barbara De la Salle
UK NEQAS Haematology, Watford, WD18 OJ, UK

Introduction

The UK NEQAS Haematology Abnormal Haemoglobins (AH) programme is designed to assess the performance and interpretation of non-molecular detection techniques in screening and diagnostic testing for the haemoglobinopathies using liquid blood specimens.

Participants analyse blood specimens from adult ‘patients’ by the methods and are expected to give a presumptive identification of any haemoglobin variant present and quantitate Hb A₂, Hb F and Hb S (if present).

Methods

The testing methods in use are predominantly high performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE).

Specimen 1806AH3 was from a carrier of a delta globin chain carrier, distributed to 332 participating laboratories.

Case Study

Clinical details described the patient as a 33 years’ old Northern European female ‘patient’ tested as part of antenatal screening. Her full blood count was normal and haemoglobinopathy screening showed a Hb A₂ variant peak (Hb A₂ prime (A₂')) resulting from the presence of a delta globin chain variant (see Figures 1 & 2). Delta globin chain carriers usually have a Hb A₂% percentage that is slightly lower than the main Hb A₂ peak. Note that a Hb A₂ variant may also be seen in carriers of an alpha globin chain variant.

Figure 1 Bio-Rad Variant II high performance liquid chromatography (HPLC) chromatogram showing a split Hb A₂. The red arrow shows Hb A₂ and the purple arrow Hb A₂'.

Results

A total 177/293 (60%) of participants who returned a haemoglobin variant identification for this ‘patient’ suggested the presence of a Hb A₂ variant. Hb A₂% results returned showed a bimodal distribution of Hb A₂% (see fig. 3), demonstrating that some participants reported the quantitation of the Hb A₂ peak only and others the sum of the Hb A₂ and Hb A₂ variant peaks.

There was a variation between users of different HPLC analysers as to whether the Hb A₂ peak was detected. Despite the existence of national standards, 32/130 UK laboratories (including 24 NHS laboratories in England) did not note the presence of a Hb A₂ variant using either coded or written comments.

This specimen was withdrawn from performance assessment for Hb A₂ quantitation due to lack of consensus in the Hb A₂% but reported for its significant educational content.

Discussions/ Conclusions

A delta globin chain variant is clinically insignificant but has the potential to confound the accurate estimation of the total Hb A₂% as it splits the Hb A₂ peak. The variation seen between participating laboratories in the quantitation and interpretation of Hb A₂% in the presence of a Hb A₂ variant raises concern about the possibility of misdiagnosis of a beta thalassaemia carrier, especially in antenatal patients.

The inter-method variation observed was related in part to the instrumentation used, as it is recognised that some HPLC instruments do not detect Hb A₂ variant peaks, and highlights the importance of understanding the limitations of the methodology in use in the laboratory.