DAkkS Deutsche Akkreditierungsstelle D-ML-13403-01-00 D-PL-13403-01-00

Überörtliche Berufsausübungsgemeinschaft
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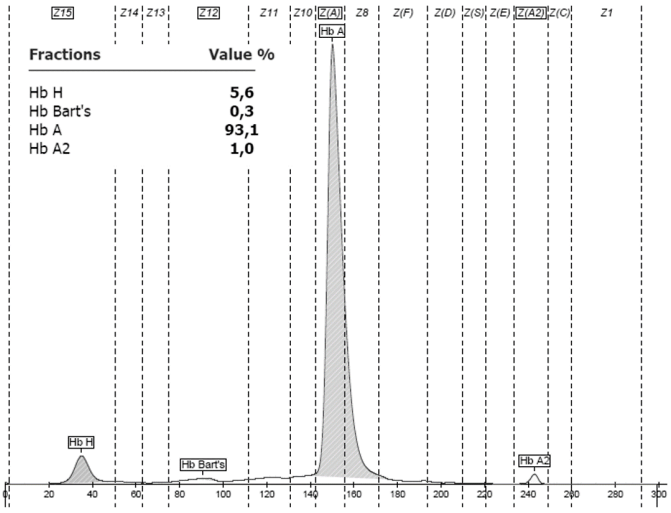
Laboratory information 1/2017

Advanced high resolution capillary electrophoresis for the detection of hemoglobinopathies

Medical background

Hemoglobinopathies are a heterogeneous group of more than 1,000 disorders, which can be categorized into two main groups: hemoglobins variants and thalassemias. The hemoglobins variants resp. abnormal hemoglobins result from an alteration in the globin protein structure, whereas the thalassemias arise from inadequate production of structurally normal globin protein. Approximately 1000 different hemoglobin variants have been described and about 300 mutations giving rise to thalassemia. The frequency of different hemoglobinopathies varies in different ethnic groups and certain hemoglobinopathies are often associated with a family history. Many hemoglobinopathies lack clinical significance whereas others are associated with severe morbidity and mortality, most notably sickle cell disease and Beta-thalassemia major.

Beta-thalassemia is a major public health problem in Egypt, as in many Mediterranean countries. It has been estimated that 1000 children out of 1.5 million live births are born per year with thalassemia major. The carrier rate in Egypt has been reported to be up to 10%. Thus, the identification and quantification of hemoglobins represent a relevant clinical tool in prenatal screening, as well as in the diagnosis and monitoring of hematological diseases and their complications.



Definitive methods, such as DNA analysis and mass spectrometry, are time-consuming and costly. Currently, biochemical detection procedures in use include alkaline agarose gel electrophoresis, cellulose acetate electrophoresis with elution followed by scanning densitometry, micro-column chromatography, and high performance liquid chromatography (HPLC). Although especially HPLC is still found in the majority of hematological guidelines for the detection of hemoglobin variants, from an analytical point of view all those techniques must to be regarded as error-prone methods.

Via HPLC, Hb H and Hb St. Barts are difficult to identify, many hemoglobin variants show similar retention times, and e. g. Hb A2 is difficult to quantify in presence of Hb C. Furthermore, HPLC provides an inaccurate separation and especially insufficient peak quantification.

By contrast, capillary electrophoresis is the latest state-of-the-art technology for the use in clinical laboratories to screen samples for hemoglobin variants and thalassemias. Here, hemoglobin fractions are separated in silica capillaries, by their electrophoretic mobility and electroosmotic flow at a high voltage. Direct detection and quantification is performed at a specific wavelength at the cathodic end of the capillary. This technique enables the efficient separation of hemoglobin fractions and detection of a large number of hemoglobin variants and thalassemias patterns. Capillary electrophoresis allows high resolution separation of the most common hemoglobin variants (e. g. Hb S, Hb C, Hb D and Hb E) presenting sharp peaks at a perfect baseline, hereby allowing a precise quantification (see figure). Even the instable Hb H and Hb St. Bart's, as seen in alpha-thalassemia, are clearly separated and easily quantified.

Method and turnaround time

Capillary electrophoresis (CAPILLARYS® 2 FLEX PIERCING, Sebia, France); once per week

Material

EDTA whole blood or newborn's cord blood sample, about 2 ml

Reference intervals & cut-offs

For children (> 12 months) and adults

Hb A:	> 96 %
Hb A2:	2.2-3.0 %
Hb F:	< 1.1 %
Atypical hemoglobins:	negativ

For newborns (< 12 months)

Individual age-specific cut-offs for Hb F

For each medical report, we supply an individual interpretation along with further diagnostic recommendations including molecular-biological analysis.

Contact

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Literature

1. Sickle Cell and Thalassemia - Handbook for Laboratories. NHS Screening Programmes, Second Edition, Sep. 2009.
2. Altinier et al. Identification and quantification of hemoglobins in whole blood: the analytical and organizational aspects of Capillarys 2 Flex Piercing compared with agarose electrophoresis and HPLC methods. Clin Chem Lab Med 2012.

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