The use of capillary electrophoresis for hemoglobinopathies diagnosis: preliminary analytical performances of CAPILLARYS 3 TERA, SEBIA

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BACKGROUND

Hemoglobinopathies are the most common monogenic diseases in human. Approximately 7% of world population is carrier and 2.7 conceptions per 1000 are affected by these pathologies. Hemoglobinopathies are qualitative (Hemoglobin variants) and/or quantitative disorders (thalassemias) of the hemoglobin. They can lead to several clinical symptoms, from benign (mild microcytosis) to the most severe (sickle cell disease, hemoglobin Fetalis) with multiple end-organ damages. According to different national and international guidelines, diagnosis of hemoglobinopathies is mainly based on Complete Blood Count and on the separation and quantification of the different hemoglobin fractions. Last points can be achieved by different separative techniques such as HPLC, IEF or more recently, Capillary Electrophoresis. Here we describe the analytical performances of the new hemoglobin diagnosis kit CAPI 3 HEMOGLOBIN(E) on CAPILLARYS 3 TERA, the high-throughput and high-volume capillary electrophoresis instrument from SEBIA.

MATERIALS & METHODS

Hemoglobin testing to capillary electrophoresis:
The CAPILLARYS 3 TERA instrument uses the principle of capillary electrophoresis (electrokinetic separation technique carried out in a tube of internal diameter lower than 100 µm filled with a buffer composed of electrolytes) in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an electric field per tube. Separation also occurs according to the electrolyte pH and ionic strength of the sample.

The CAPILLARYS 3 TERA instrument has six capillary fractions in parallel allowing 12 simultaneous analyses for hemoglobin quantification in a whole blood sample. Minimum sample volume required is 200 µL. A sample dilution with terminating solutions is prepared and injected by aspiration at the anodic end of the capillary. A high voltage power separation is then performed and direct detection of the hemoglobin fractions is made at the cathodic end of the capillary at 660 nm (hemoglobin absorbance wavelength). Direct detection provides accurate relative quantification of individual hemoglobin fraction.

Limit of detection:
Limit of detection of the CAPI 3 Hemoglobin(E) method was assessed by analyzing samples with Hb variants (Hb S, C, D, E, other Hb variants), all at heterozygous state.

RESULTS

The CAPILLARYS 3 TERA is able to pick up all major common Hb fractions (Hb S, F, C, D, E) and the CAPILLARYS 2 TERA, as well as the CAPILLARYS 2 Flex Piercing with HEMOGLOBIN(E) method (reference method). The overall impression is good for all Hb fractions considered, with CV lower than 6%. Results of reproducibility studies are summarized in the Table 1.

The correlation between CAPILLARYS 3 TERA and CAPILLARYS 2 Flex Piercing is excellent (r = 0.990), showing perfect agreement in measuring Hb A, Hb A2, Hb F and major common Hb variants (Hb S, C, D and E) (Figure 2).

CONCLUSION

CAPILLARYS 3 TERA from SEBIA is a multiparametric capillary electrophoresis instrument which can analyze serum proteins and perform their immunotyping. It is also able to manage whole blood on capped tube at a high throughput (up to 70 samples/hour), allowing the measurement of Hb A1c. In addition, the CAPILLARYS 3 TERA and HEMOGLOBIN(E) method allows an accurate separation and quantification of the hemoglobin fractions. Its overall analytical performances make it a solution of choice for any type of laboratories that would like to perform hemoglobinopathies diagnosis at high degree of confidence.