# A NEW FAST HEMOGLOBIN ELECTROPHORESIS PERFORMED ON SEBIA CAPILLARYS SYSTEM

G. Hennache, F. Robert, J.B. Clément, D. Simonin and G.Barouh
Sebia, Parc Technologique Léonard de Vinci - CP 8010 Lisses - 91008 Evry Cedex France

sebia

## ABSTRACT

Hemoglobin electrophoresis is a conventional method used for the detection and quantification of hemoglobin abnormalities such as the most commonly found hemoglobin variants and thalassemias. This test has been recently adapted to the SEBIA capillary electrophoresis system, CAPILLARYS™ to offer complete automation of hemoglobin electrophoresis including the following steps: preparation of the hemolysate from unwashed RBC's, sample migration, direct detection, and quantification/identification of the hemoglobin fractions.

## PRINCIPLE OF THE TEST

The hemoglobin spatial structure and other molecular properties depend on the nature and the sequence of the amino acid composition. Mutation by amino acid substitution is responsible for hemoglobin variants.
These variants have different surface charges and consequently, different electrophoretic mobilities dependent on the pH and ionic strength of the assay buffer.
The resulting qualitative or structural abnormalities are referred to as hemoglobinopathies. Decreased synthesis of one of the hemoglobin chains leads to quantitative or regulation abnormalities, referred to as thalassemias.
The CAPILLARYS system uses the principle of capillary electrophoresis in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer.

## PROCEDURE

The analysis is performed on packed and unwashed red blood cells from settled or centrifuged blood collected on anticoagulant tubes. The CAPILLARYS has capillaries functioning in parallel allowing seven (7) simultaneous hemoglobin analyses.
All procedural steps are fully automated including:
- Bar code reading of sample tubes and sample racks,
- Sample hemolysis and dilution from primary tubes (without plasma) into dilution segments,
- Injection of hemolyzed samples into the capillaries for separation,
- Hemoglobin separation at 10,000 V for 8 min at a tempe rature of 34°C controlled by a Peltier system,
- Direct detection of the separated hemoglobins at 415 nm.

Performances of the Capillarys Hemoglobin test were compared to HLPC (Bio-Rad Variant I) for the determination and quantification of hemoglobin A2 and F linked to thalassemia diseases.

Another study was conducted to compare the detection of hemoglobin variants between the following three (3) techniques:
1. HPLC (Bio-Rad Variant I, β-thalassemia Short Program),
2. Agarose electrophoresis (Sebia Hydrasys system, Hydragel 15 Hemoglobin(e)),
3. Capillary electrophoresis (Sebia CAPILLARYS system, CAPILLARYS Hemoglobin(e)).

## MATERIAL

Quantitative determination of Hb A2:
The levels of Hb A2 were measured in 73 blood samples with normal and elevated levels of Hb A2 both by electrophoretic separations obtained with CAPILLARYS Hemoglobin(e) procedure and HPLC system from Bio-Rad for Hb A2 quantification. The measured values of Hb A2 from both procedures were analyzed by a linear regression statistical procedure.

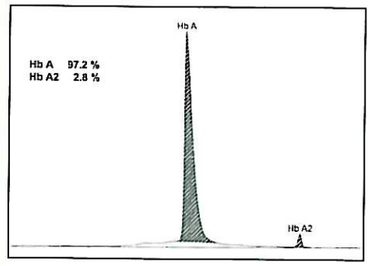

figure 1: Normal sample

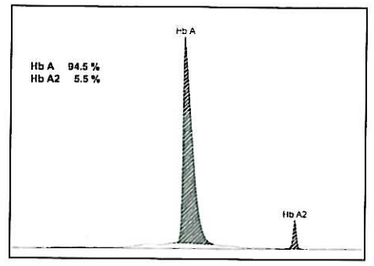

figure 2: Beta thalassemia

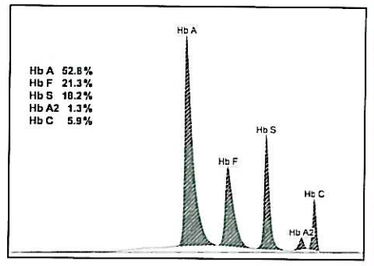

figure 3: AFSC control

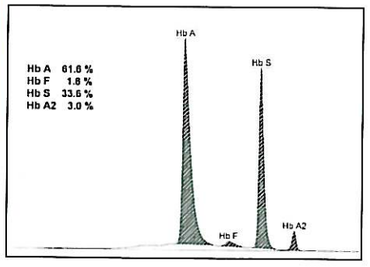

figure 4: Heterozygous A/S

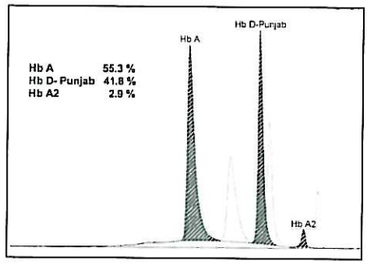

figure 5: Heterozygous A/D overlayed with AFSC control

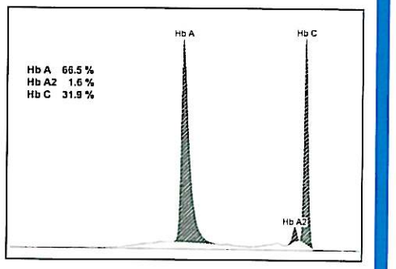

figure 6: Heterozygous A/C

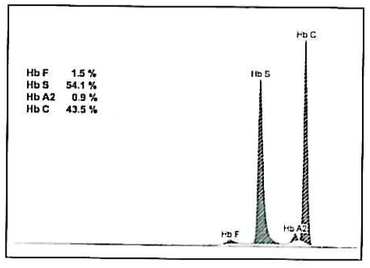

figure 7: Heterozygous S/C

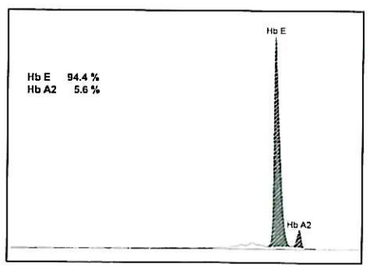

figure 8: Homozygous E

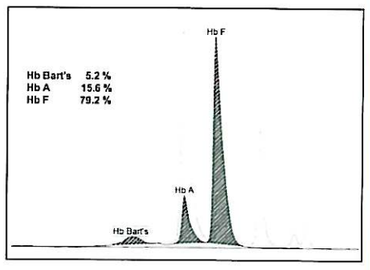

figure 9: Alpha thalasemia with Hb Bart's overlayed with AFSC control

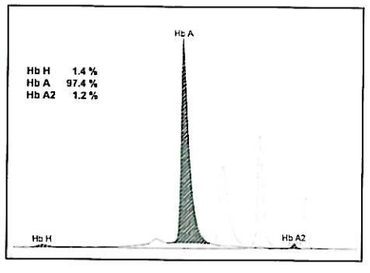

figure 10: Alpha thalasemia with Hb H overlayed with AFSC control

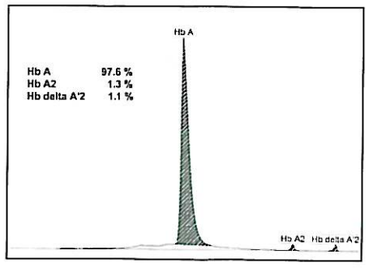

figure11: Delta A'2 variant

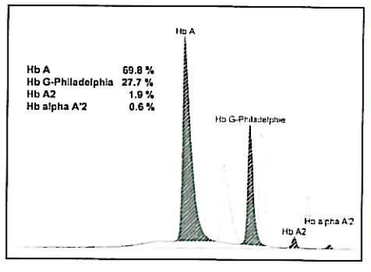

figure 12: Heterozygous A/G Philadelphia overlayed with AFSC control

Quantitative determination of Hb F:
The levels of Hb F were measured in 74 blood samples with normal and elevated levels of Hb F both by electrophoretic separations obtained with Capillarys Hemoglobin(e) procedure and HPLC system from Bio-Rad for Hb F quantification. The measured values of Hb F from both procedures were analyzed by a linear regression statistical procedure.

Detection of hemoglobin abnormalities:
75 different blood samples with hemoglobin variants, such as hemoglobins S, C and E were analyzed with both the CAPILLARYS Hemoglobin(e) procedure and HPLC system from Bio-Rad.

For the CAPILLARYS, either a normal or a pathological abnormal control (with elevated A2 level or with A, F, S and C fractions) was included for the fraction identification.

Quantitative determination of Hb S:
The levels of Hb S were determined in 43 samples by both the CAPILLARYS Hemoglobin(e) procedure and HPLC system from Bio-Rad. The samples were a mix of heterozygous and homozygous patients. The measured values of Hb S from both procedures were analyzed by a linear regression statistical procedure.

## RESULTS

Quantification of Hb A2:
The correlation coefficient for Hb A2 between the HPLC system and CAPILLARYS is **0.95**.

Quantification of Hb F:
The correlation coefficient for Hb F between the HPLC system and CAPILLARYS is **0.99**.

Detection of hemoglobin abnormalities:
Several variants were tested during the study including heterozygous and homozygous Hb S, Hb C, Hb E samples. Additionally Hb S/C, Hb D-Punjab, Hb G-Philadelphia, Hb delta A'2, Hb H, and Hb Bart's were analyzed. All of those hemoglobins were fully detected and identified with the 3 techniques. Patterns obtained with CAPILLARYS are shown in figures from 1 to 12.

During this study other variants such as J-Mexico, Hope, Winnipeg, Korle-Bu, O-Arab, J-Providence, N-Baltimore and Camperdown hemoglobins were also easily detected with Capillarys (results not shown).

The correlation coefficient for Hb S measurement between the HPLC system and CAPILLARYS is **0.99**. This coefficient clearly demonstrates that treatment of Hb S patients can be reliably monitored utilizing the Sebia CAPILLARYS System.

## CONCLUSION

The main advantages of the CAPILLARYS Hemoglobin assay are:
- Enhanced resolution and focalization of all hemoglobin fractions allowing an excellent separation of variants plus an accurate quantification of A2, essential for the diagnosis of thalassemias,
- Clear separation and focalisation of F hemoglobin between the A and S fractions allowing precise quantification of F,
- A distinct separation of hemoglobins C and E from the A2 fraction facilitating identification and quantification,
- Utilization of stored reference curves as overlays facilitating variant identification such as easily identifying closely migra ting hemoglobins S, D, and G-Philadelphia,
- Excellent correlation with HPLC, including Hb F and HbA2 quantitative results,
- An excellent separation/detection of Hb A2 variants,
- Hb H and Bart's can be identified with confidence,
- A high throughput: 34 samples/hour.