Sebia CAPILLARYS 2 capillary electrophoresis: useful tool in the algorithm of screening and characterization of abnormal hemoglobin.

Jean Riou[1], Christian Godart[1], Henri Wajcman[2], Josiane Bardakdjian-Michau[1]
[1] Genetic Biochemistry Department, Henri Mondor Hospital, Créteil, FRANCE
[2] U955 INSERM Unit, Henri Mondor hospital, Créteil, FRANCE

INTRODUCTION

Capillary electrophoresis (CE) has become a technique of choice for the diagnosis of hemoglobinopathies in routine laboratories. While by HPLC, the retention time is used for the presumptive identification of the variants, the migration time in CE is usually not exploited and the presumptive identification of variants is based on their electrophoretic mobilities in migration "zones" defined by the manufacturer. With this work, we have studied the reproducibility of the migration positions of several common hemoglobin variants (Hb S, Hb C, Hb D-Punjab, Hb E and Hb Hope) by CE, in order to check if the "migration time" of a variant can be used to obtain a better discrimination between variants with close mobilities, and thus to refine the presumptive identification of variants.

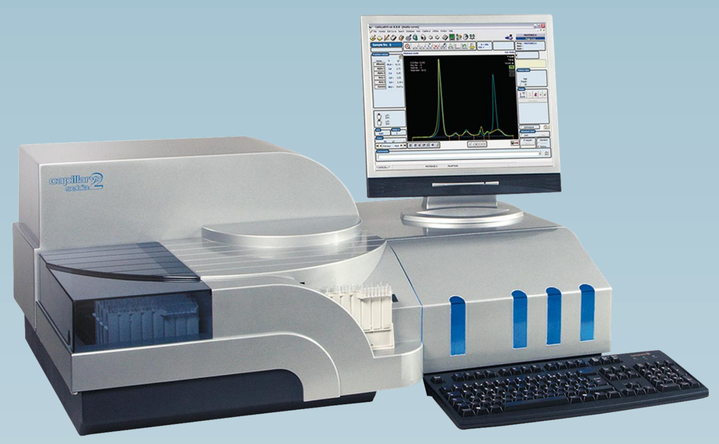

Figure 1: The electrophoresis apparatus CAPILLARYS 2 from Sebia

MATERIAL AND METHODS

Hemoglobin capillary electrophoresis was performed on the CAPILLARYS 2 instrument (Sebia, France) **(Fig.1)** with the CAPILLARYS HEMOGLOBIN(E) kit and Phoresis CORE software (version ≥ 6.50), according to manufacturer's recommendations. The intra- and inter-assay reproducibility of the variants' migration positions was assessed on a selection of 7 fresh blood samples: 1 normal sample (A/A), 5 samples with a common variant at the heterozygous state (1 A/S, 1 A/C, 1 A/D, 1 A/E, 1 A/Hope) and 1 normal control (Sebia, ref 4774). For the intra-assay reproducibility, each sample was analyzed on the 8 capillaries simultaneously, with 2 different buffer lot numbers (n=16). The inter-assay reproducibility was assessed after repeating the analysis of each sample 6 times, during consecutive series of analysis, using with 2 different buffer lot numbers (n=12). Inter-individual repeatability of the variants' migration positions was assessed by selecting 61 samples presenting a common hemoglobin variant: 20 A/S, 10 A/C, 10 A/E, 10 A/D and 11 A/Hope. These samples were analyzed randomly over several days. Mean values, standard deviations and coefficients of variation for each reproducibility study were calculated for the migration position of each hemoglobin variant. Following this reproducibility study, the electrophoretic mobilities of more than 200 rare hemoglobin variants from our blood samples library were determined on CAPILLARYS 2.

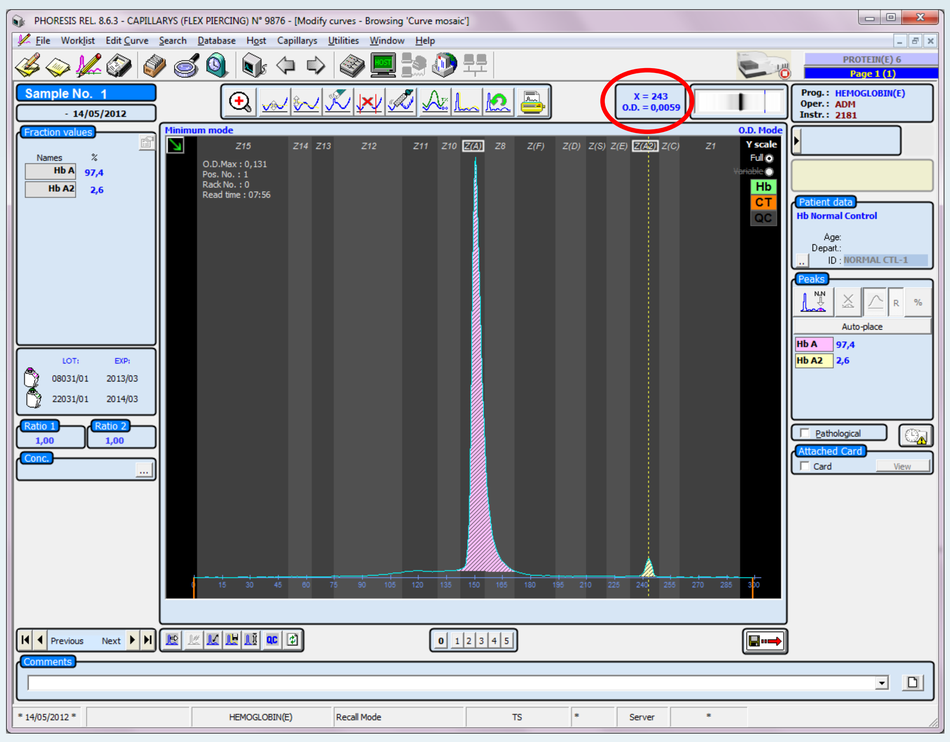

Figure 2: Screenshot of the interpretation and validation software, PHORESIS, allowing the visualization of the fractions' migration position (red circle)

RESULTS

On CAPILLARYS 2, positions of Hb A and Hb A2 are normalized, Hb A always migrating in position 150 and Hb A2 always migrating in position 243 **(Fig. 2)**. Note that the units used to determine the migration position on the horizontal axis (X) do not correspond to migration times, these are arbitrary units defined by the manufacturer (0≤X≤300). The reproducibility of the variants' migration positions is excellent, with CVs below 0.49% and 0.39% for the intra- and inter-assay reproducibility, respectively **(Tables 1 & 2)**. The inter-individual repeatability of the variants' migration positions is also excellent with CVs below 0.62% **(Table 3)**. Having demonstrated the very good reproducibility of the migration positions of common variants, we have determined the migration positions of more than 200 rare hemoglobin variants on CAPILLARYS 2 in order to determine the electrophoretic mobilities of these variants.

	Hb Hope	Hb D	Hb S	Hb E	Hb C
Min	132	207	213	227	252
Max	134	208	214	228	253
Mean	132,8	207,1	213,5	227,7	252,2
SD	0,66	0,34	0,52	0,48	0,40
CV	0,49%	0,16%	0,24%	0,21%	0,16%

Table 1: Intra-assay reproducibility of the variants migration positions on CAPILLARYS 2 (n=16)

	Hb Hope	Hb D	Hb S	Hb E	Hb C
Min	132	207	214	227	252
Max	134	209	214	228	253
Mean	133,1	207,9	214,0	227,8	252,2
SD	0,51	0,51	0,00	0,45	0,52
CV	0,39%	0,25%	0,00%	0,20%	0,21%

Table 2: Inter-assay reproducibility of the variants migration positions on CAPILLARYS 2 (n=12)

	Hb Hope (n=11)	Hb D (n=10)	Hb S (n=20)	Hb E (n=10)	Hb C (n=10)
Min	132	207	212	227	251
Max	134	209	216	228	252
Mean	133,0	207,3	213,9	227,9	251,8
SD	0,63	0,67	1,33	0,32	0,42
CV	0,48%	0,33%	0,62%	0,14%	0,17%

Table 3: Inter-individual repeatability of the variants migration positions on CAPILLARYS 2

CONCLUSION

The migration positions of the hemoglobin variants on CAPILLARYS 2 are perfectly reproducible, for both variants with anodic migration (Hb Hope) and cathodic (Hb C). This study demonstrates that the migration position, more precise than the migration "zones" proposed by the manufacturer, can be exploited for the presumptive identification of a variant, with an uncertainty of ± 1 on the horizontal axis. An electrophoretic mobilities mapping of more than 200 rare variants on CAPILLARYS 2 has been established and is now in our laboratory a very useful tool in the algorithm of screening and characterization of hemoglobin abnormalities.