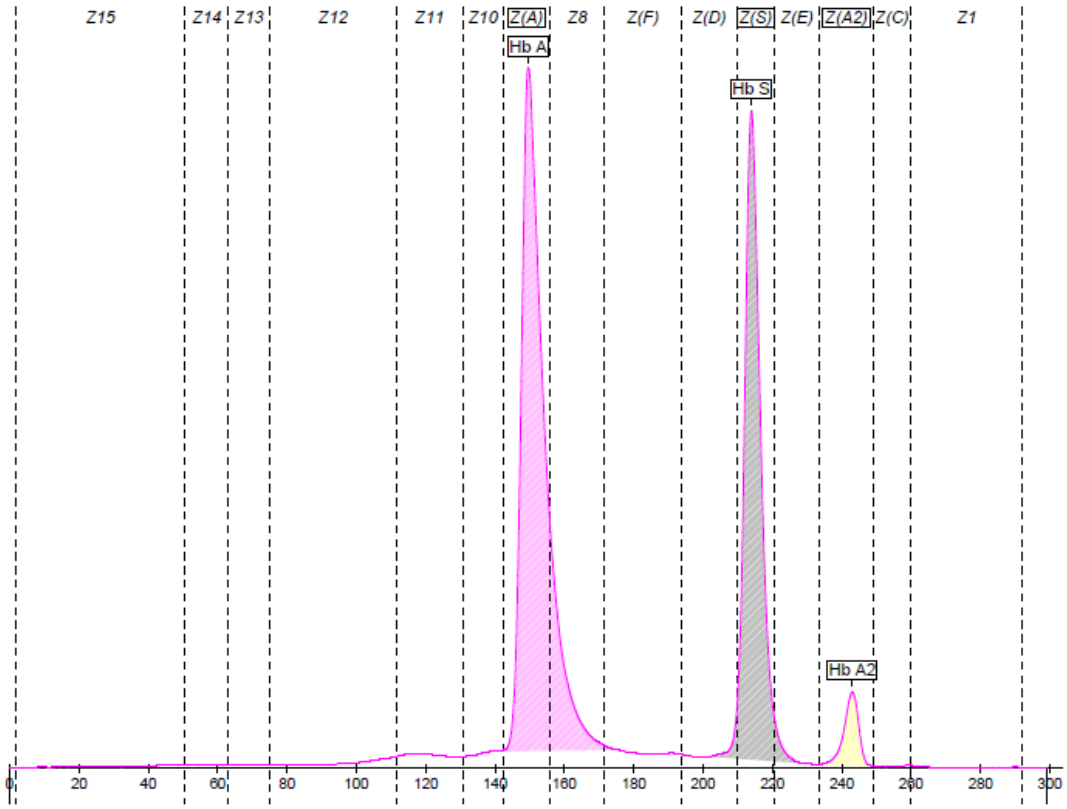
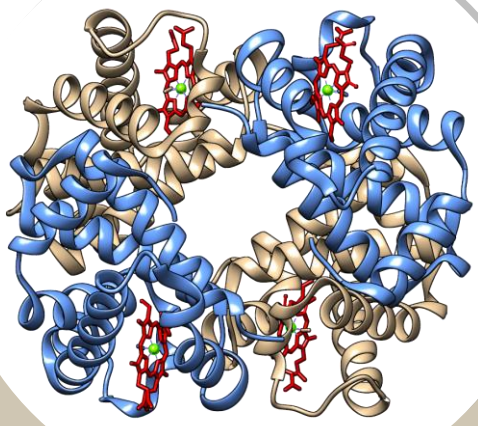
META - ANALYSIS





HEMOGLOBIN

High Resolution Separation

By SEBIA Capillary Electrophoresis

Publications & Reports 2011-2015

sebia

SEBIA Educational Library
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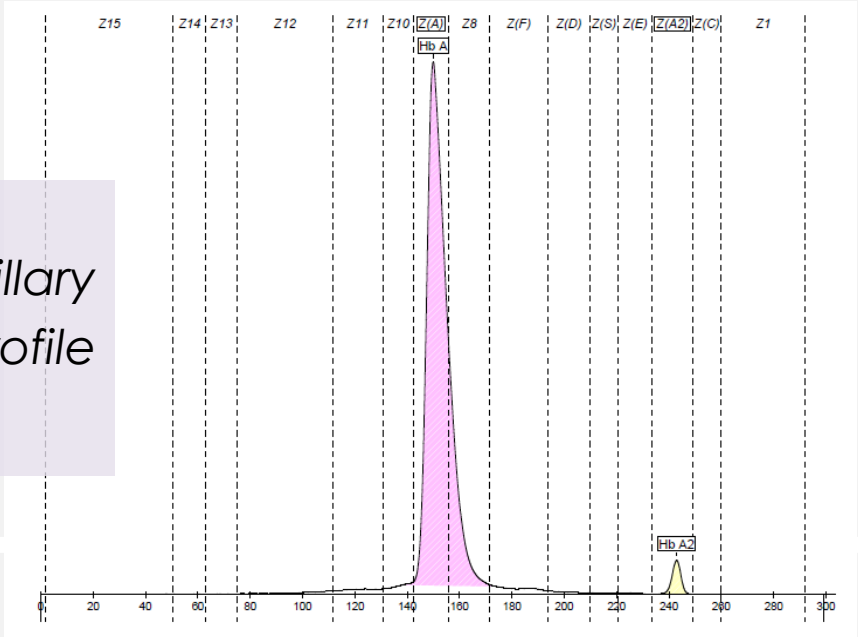
The hemoglobin (Hb) analysis by Sebia capillary electrophoresis was made first available on CAPILLARYS 2 in 2004. This technological breakthrough was then deployed on MINICAP (2008), CAPILLARYS 2 Flex Piercing (2010) and finally on MINICAP Flex Piercing (2011).

Since more than ten years, many scientific evidences have been generated. This document has been issued from a bibliographical review of analytical performances already published, communicated or currently in press.

The Sebia Hb capillary electrophoresis provides to all laboratories a suitable technique for the identification and the quantification of common and unusual hemoglobinopathies and thalassemias, at a high resolution and high throughput.

Hemoglobin capillary electrophoresis profile



CAPILLARYS 2

Multicenter validation of fully automated capillary electrophoresis method for diagnosis of thalassemias and hemoglobinopathies in Thailand

Sangkitporn S, Sangkitporn SK, Tanjatham S, Suwannakan B, Rithapirom S, Yodtup C, Yowang A, Duangruang S.
Ref. #13, page 41.

Precision level	Hb E	
	Percent Hb E	Percent CV
Within run precision		
Quality control material		
Normal Hb A_2 control	-	-
AFSC control	-	-
Between run precision		
Quality control material		
Normal Hb A_2 control	-	-
AFSC control	-	-
EDTA blood sample		
Normal subject	-	-
β-thalassemia carrier	-	-
Hb E carrier	29	3.02
β-thalassemia/ Hb E	38.86	3.03
β-thalassemia homozygote	-	-
Inter-laboratory precision		
EDTA blood sample		
Normal subject	-	-
β-thalassemia carrier	-	-
Hb E carrier	22.4	4.46
Hb E carrier	22.25	2.45

Table 1. Precision of Hb A2, Hb F and Hb E quantification using Capillarys 2 system

Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies

Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R.
Ref. #12, page 41.

"The between-run consistency of the CE was evaluated on 17 consecutive runs of the standard preparation containing HbA, HbF, HbS, and HbC. The mean, SD, and coefficient of variation (CV) were as follows: HbA, 28.1% (SD, 0.54%; CV, 1.91%); HbF, 31.0% (SD, 0.43%; CV, 1.39%); HbS, 30.8% (SD, 0.49%; CV, 1.60%); and HbC, 10.2% (SD, 0.28%; CV, 2.76%)."

CAPILLARYS 2 FLEX PIERCING

Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies

Greene DN, Pyle AL, Chang JS, Hoke C and Lorey T.
Ref. #5, page 40.

"The between run variability was evaluated over 12 consecutive runs and 2 buffer lots using 3 different control materials all of which contained Hb A and Hb A2."

Table 1
Interassay variability observed for the most common hemoglobin species using the Capillarys Flex CE. QC 1, 2, and 3 denote different levels and manufacturers of quality material, as described in Materials and methods section.

	Hb A QC 1	Hb A QC 2	Hb A QC 3	Hb A2 QC 1	Hb A2 QC 2	Hb A2 QC 3	Hb F QC 1	Hb F QC 2	Hb S QC 1
Mean (%)	97.4	96.8	55.3	2.6	2.3	4.9	0.97	9.9	29.9
SD	0.07	0.12	0.5	0.07	0.08	0.17	0.07	0.18	0.37
CV (%)	0.1	0.1	0.9	2.6	3.4	3.5	7.1	1.8	1.2

Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in multi-ethnic populations

Van Delft P, Lenters E, Bakker-Verweij M, de Korte M, Baylan U, Harteveld CL, Giordano PC.
Ref. #1, page 40.

"All machines tested were able to identify the common carriers of HbS, HbC, HbE, HbD-Punjab, HbO-Arab and HbH disease."

Putative genotype	VARIANT II *N*/score	VARIANT II Name peak	Capillarys *N*/score	Capillarys Name peak
HbA/S	30/30	S window	30/30	S-zone
HbS/S	6/6	S window	6/6	S-zone
HbA/C	6/6	C window	6/6	C-zone
HbC/C	2/2	C window	2/2	C-zone
HbA/E	6/6	A_2 window	6/6	E-zone
HbE/E	3/3	A_2 window	3/3	E-zone
HbA/D-Punjab	19/19	D window	19/19	D-zone
HbA/O-Arab	1/1	Unknown	l/l	Unknown
HbH disease	4/4	Peak seen, not named	4/4	H-zone

NT, not tested.

Table 1. Degree of sensitivity for common Hb S, C, E, D-Punjab, HbO-Arab and HbH genotypes on the different devices

Comparison of two methods for the quantification and identification of hemoglobin variants

Higgins T, Mack M, Khajuria A.
Ref. #3, page 40.

"In this study 94 heterozygous and 13 homozygous HbS, 27 HbD Punjab trait, 26 HbE trait and 22 HbC trait samples were correctly identified by the Capillarys 2 system using the combination of HPLC and electrophoresis at alkaline and acid pH as the reference method. In addition the Capillarys 2 correctly identified 2 Hb Lepore, 5 HbH, 6 HbJ, 2 HbO Arab and one each of hemoglobins Q Thailand, Q India and G Norfolk."

The range of hemoglobin A(2) in hemoglobin E heterozygotes as determined by capillary electrophoresis

Mais DD, Gulbranson RD, Keren DF.
Ref. #11, page 41.

"CE has the ability to completely separate HbA2 from HbE, a distinction possible by only one of the currently available clinical methods of HPLC and not possible by traditional gel electrophoresis."

Thalassaemias: detection, characterisation and laboratory interpretation

Youssef E.
Ref. #24, page 42.

"To date, the only routine method able to separate HbA2 from HbE and Hb Lepore is probably capillary electrophoresis."

Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies

Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R.
Ref. #12, page 41.

"Whereas only 3 cases containing HbE were encountered during this prospective study, the complete separation of HbA2 from HbE by CE in all 3 compared with the lack of measurable HbA2 by HPLC deserves note. (…) While the HPLC attempts to distinguish HbA2 (peak 13), the overlap with HbE (peak 12) is too great for a reliable estimate. Our interpretive report for the HPLC notes that the HbE value includes HbA2. However, the CE pattern in this same case demonstrates a clean separation of HbA2 from HbE."

"In addition to the 39 cases containing HbS, 14 cases containing HbC and 3 cases containing HbE variants correctly identified by both methods included the following: 2 cases containing HbS and HbC, 2 cases of variant HbA2, 2 cases of HbD-Los Angeles (Punjab) trait, 1 HbF variant, 1 case of HbG-Philadelphia (α) trait, 1 case of HbS-G Philadelphia, and 1 case of Hb Lepore. In addition, CE detected 1 case of Hb Athens/Waco, whereas the screening HPLC did not."

Multicenter validation of fully automated capillary electrophoresis method for diagnosis of thalassemias and hemoglobinopathies in Thailand

Sangkitporn S, Sangkitporn SK, Tanjatham S, Suwannakan B, Rithapirom S, Yodtup C, Yowang A, Duangruang S.
Ref. #13, page 41.

"Presumptive identifications of the common Hb variants present in the Thai population, namely, Hb E and Hb Constant Spring (CS), were clearly possible due to their different positions from those of Hb A, Hb A2 and Hb F in the electrophoregrams of Capillarys 2 System (Fig 2B and C). Three rare Hb variants (Hb J-Bangkok, Hb G-Makassar and Hb C) were also detected (Fig 2D, E and F)."

Group	N	Hb pattern	Interpretation	Degree of agreement
1	116	A_2A	Normal Hb typing	100%
2	66	A_2A	β-thalassemia carrier	100%
3	2	A_2FA	β-thalassemia homozygote	
4	120	EA	Hb E carrier	100%
5	30	EF	β-thalassemia/ Hb E	100%
6	25	A_2ABart'sH	Hb H disease	100%
7	50	EE	Hb E homozygote	100%
8	1	Abnormal Hb	Hb J Bangkok carrier	100%
9	1	Abnormal Hb	Hb G Makassar carrier	100%
10	1	Abnormal Hb	Hb C carrier	100%

Table 2. Capillarys 2 System results obtained from blood samples of thalassemias and hemoglobinopathies frequently observed in Thailand. Degree of agreement represents percent agreement between final interpretation of Capillarys 2 System and those of HPLC and LPLC techniques used as comparative methods.

RESOLUTION/VARIANTS DETECTION

Novel hemoglobin UKB demonstrates the importance of using different methods of detection

Zur B, Stoffel-Wagner B, Ludwig M.
Ref. #7, page 40.

"In a 73-year old male patient we detected a novel hemoglobin anomaly, termed by us Hemoglobin UKB, which cannot be detected by chromatography (Variant II, Bio Rad) but which shows a capillary electrophoresis fraction of 50.9% (Capillarys, Sebia)."

Detection of Hb Constant Spring by a capillary electrophoresis method

Liao C, Zhou JY, Xie XM, Li J, Li R, Li DZ.
Ref. #22, page 42.

"Automated high performance liquid chromatography (HPLC) and Sebia Capillarys 2, a capillary electrophoresis method, were applied to blood samples from 21 individuals with Hb CS trait. Of the 21 cases, all (100%) were detected by capillary electrophoresis, whereas only 16 (76.2%) were detected by HPLC. We concluded that the Sebia Capillarys 2 is the preferred method for Hb CS screening."

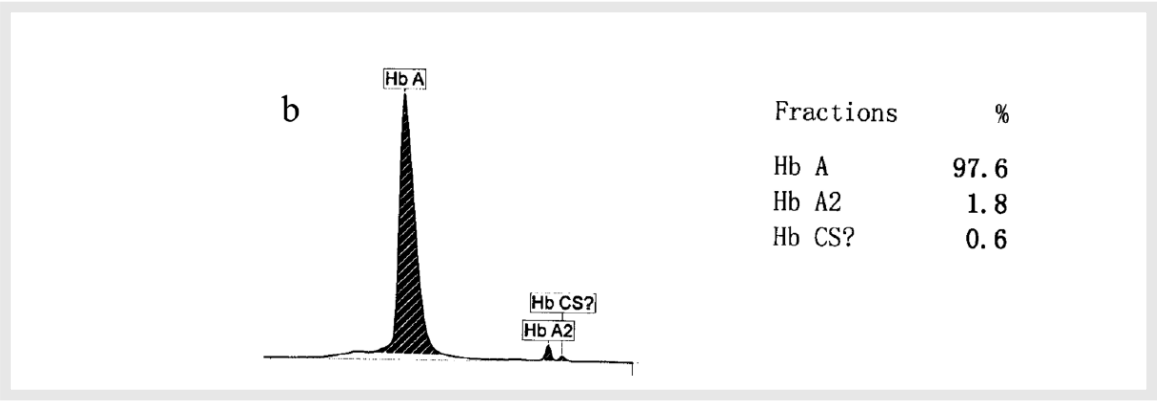


FIGURE 1. (b) Sebia Capillarys 2 pattern of a Hb CS trait patient.

Screening for Hb Constant Spring in the Guangdong Province, South China, using the Sebia capillary electrophoresis system

Liao C, Zhou JY, Xie XM, Li DZ.
Ref. #9, page 41.

"As shown in this study, the lowest level of Hb CS identified by the Capillary 2 system in adult heterozygotes was 0.1%, which might be too low to be detected by other routine methods for Hb analysis such as traditional electrophoresis or high performance liquid chromatography (6,10). We believe that all subjects with Hb CS trait present in our random cohort have been identified using the Sebia Capillarys 2 system."

RESOLUTION/VARIANTS DETECTION

Higher sensitivity of capillary electrophoresis in detecting hemoglobin A2' compared to traditional gel electrophoresis

Oleske DA, Huang RS, Dasgupta A, Nguyen A, Wahed A.
Ref. #23, page 42.

"In capillary electrophoresis, Hb A2' is detected by its presence in zone 1, Hb S is seen in zone 5, Hb C in zone 2, and Hb G in zone 6. Thus, capillary electrophoresis simplifies HbA2' detection because the HbA2' elutes in different windows from the major hemoglobins."

"We believe that capillary electrophoresis allows for better detection of Hb A2' than gel electrophoresis and HPLC do."

Comparison of capillary electrophoresis and high performance liquid chromatography for detection and quantification of hemoglobin New York

You-Qiong L, Hui-Ping H, Zhi-Zhong C, Lin Z, Liang L, Gui-Fang Q, Yun M.
Ref. #4, page 40.

"In conclusion, Sebia CAPILLARYS 2 system (CE) correctly identified all samples with Hb New York which was not detected by HPLC. Furthermore, HPLC has to rely on published retention time information when rare variants occur, while CE provides an integrated library of Hb variants to make the analysis of the results more convenient. Thus, CE presents advantages over HPLC, at least for the detection of Hb New York."

Rare Hb variant, not identified by HPLC, is identified by Capillary electrophoresis – Case study

Filon D, Rotschild M, Temin F, Zalman L, Kops Z, Vika, Aviv S.
Ref. #18, page 42.

"Whole blood sample was analyzed by Capillary electrophoresis (Sebia) for Hb variants. The results indicated the presence of 19% variant in D Zone (zone 6).(…) A routine HPLC (Variant II, Bio-Rad) was performed and came out negative. (…) Literature evidence suggested that the identity of the variant is P-Nilotic, a rare case of Beta –Delta rearrangement. (…) The P-Nilotic identity was confirmed by Sequencing analysis of the PCR product."

CORRELATION

CAPILLARYS 2

The range of hemoglobin A(2) in hemoglobin E heterozygotes as determined by capillary electrophoresis

Mais DD, Gulbranson RD, Keren DF.
Ref. #11, page 41.

"The higher percentage resulting from combining the HbE and HbA2 by the CE technique in our study compared with our HPLC technique likely relates to the underestimation of HbE due to separation of the glycated fraction of HbE by the HPLC technique (the glycated fraction in that technique is included with HbA). However, the CE technique does not separate glycated or other posttranslational products, thereby providing a more complete measurement of HbE as reported previously for HbS and HbC."

Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies

Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R.
Ref. #12, page 41.

"There was good agreement between HPLC and CE in measuring the percentage of HbS in the 39 samples that contained this variant—a small, but consistently higher value was found in samples measured by CE (mean, 40.6%; SD, 18.9%) than in samples evaluated by HPLC (mean, 38.4%; SD, 18.9%). This small increase in HbS in samples evaluated by HPLC may have reflected different handling of glycated fractions of HbS by these methods."

CAPILLARYS 2 FLEX PIERCING

Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies

Greene DN, Pyle AL, Chang JS, Hoke C and Lorey T.
Ref. #5, page 40.

"Hb S was detected in 24 patients by both techniques. The 2 assays had good agreement (bias=0.93; r=0.996) with the mean for the HPLC and CE 45.7% (SD, 20.5%) and 46.7% (SD, 19.3%), respectively. Hb C was detected in 9 patients by both techniques. There was fair inter-assay agreement (bias=−0.81; r=0.967) with the mean for the HPLC and CE 36.6% (SD, 4.78%) and 35.8% (SD, 5.75%), respectively."

CORRELATION

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

Altinier S, Varagnolo M, Zaninotto M and Plebani M.
Ref. #15, page 41.

"On comparing the methods for HbS quantification the following results were obtained (…)."

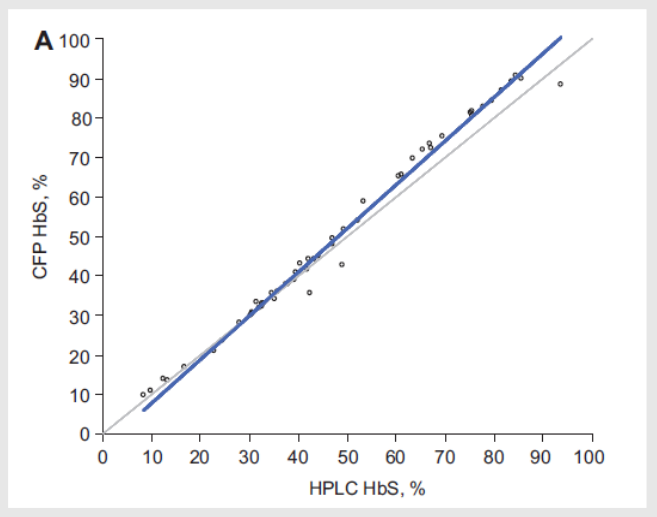


Figure 3. Passing and Bablok regression of HbS measurements obtained in Capillarys 2 flex piercing vs. HPLC. (A) all samples tested (n = 59): CFP = 1.10×HPLC– 3.24.

Integration of Capillarys 2 Flex Piercing (Sebia) in the daily practice of a specialized pathology laboratory

Guis L, Chaumiera A, Le Galla V, Havreza S.
Ref. #20, page 42.

"Furthermore, we have observed a really good correlation between the two techniques for the quantification of the variants HbS and HbC."

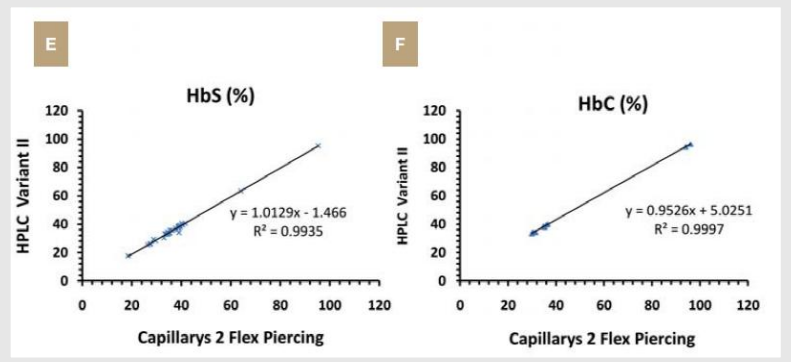


Figure 4 – Correlation of the different hemoglobin fractions between the Capillarys 2 Flex Piercing and the HPLC (Variant II Bio-Rad).

Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in multi-ethnic populations

Van Delft P, Lenters E, Bakker-Verweij M, de Korte M, Baylan U, Harteveld CL, Giordano PC.
Ref. #1, page 40.

"The degree of sensitivity for the structural mutations, for the carriers of high HbA2 β-thal and HbH disease was 100% on all devices (…)."

	VARIANT II		Capillarys		HA 8160		G7		UItra2	
Putative genotype	*N*/score	Name peak	*N*/score	Name peak	*N*/score	Name peak	*N*/score	Name peak	*N*/score	Name peak
HbH disease	4/4	Peak seen, not named	4/4	H-zone	4/4	P1 peak	4/4	Peak seen, not named	2/2	?*

NT, not tested.
*Eventually commented as 'consisted with' or unspecified.

Table 1. Degree of sensitivity for common Hb S, C, E, D-Punjab, HbO-Arab and HbH genotypes on the different devices

Comparison of two methods for the quantification and identification of hemoglobin variants

Higgins T, Mack M, Khajuria A.
Ref. #3, page 40.

"It is noted that the Biorad method does not identify or quantitate the HbH whereas the Capillarys does."

Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies

Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R.
Ref. #12, page 41.

"we have noted that HbH and Hb Bart's are more readily detected and measured by CE than by the HPLC method."

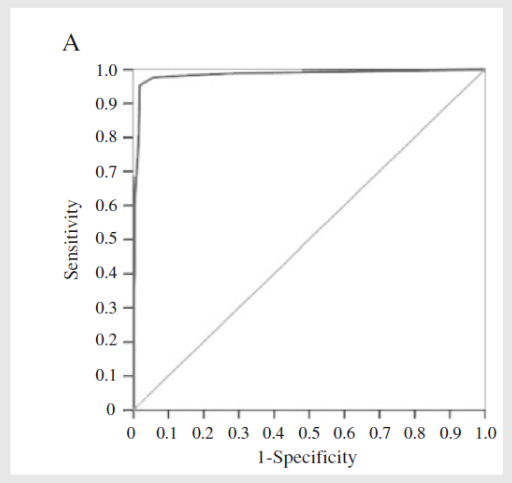
Multicenter validation of fully automated capillary electrophoresis method for diagnosis of thalassemias and hemoglobinopathies in Thailand

Sangkitporn S, Sangkitporn SK, Tanjatham S, Suwannakan B, Rithapirom S, Yodtup C, Yowang A, Duangruang S.
Ref. #13, page 41.

"In Hb H disease, Hb Bart's and Hb H were clearly separated from each other and could be readily quantitated."

Quantitative analysis of Hb Bart's in cord blood by capillary electrophoresis system

Munkongdee T, Pichanun D, Butthep P, Klamchuen S, Chalermpolprapa V, Winichagoon P, Svasti S, Fucharoen S.
Ref. #17, page 42.



B

% Hb Bart's	Sensitivity (%)	Specificity (%)	Efficiency (%)
0.1	97.73	94.40	95.08
0.2	95.45	98.23	97.66
0.3	81.82	98.53	95.08
0.4	75.00	98.82	93.91
0.5	60.23	99.71	91.57

Fig.3. The ROC analysis of α-thalassemia 2 prediction in newborns by Hb Bart's level
A The ROC curve of α-thalassemia 2 prediction at 0.2% Hb Bart's.
B The ROC analysis of α-thalassemia 2 prediction by 0.1–0.5% Hb Bart's

"The automated CE, developed for Hb fraction separation and quantitation, can measure Hb Bart's and Hb H directly from the electrophoregrams by the software."

"The ROC analysis showed that the cutoff at 0.2% Hb Bart's provided 95.45% sensitivity, 98.23% specificity, and 97.65% efficiency for α-thalassemia 2 heterozygote screening. This indicated that Hb Bart's level of 0.2% can be used as a cut-off point for α-thalassemia diagnosis in newborns."

Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies

Greene DN, Pyle AL, Chang JS, Hoke C and Lorey T.
Ref. #5, page 40.

"Similarly, using HPLC, Hb Bart's and Hb H elute in the void volume and therefore can only be distinguished when their concentration is very high (>~5%), and when they are visualized, they cannot be quantified. CE can detect and quantify these variants even at concentrations of ~1%. Moreover, bilirubin can elute in the void volume, and can be mistaken for Hb H and/or Bart's [24]. Bilirubin does not interfere with CE, making detection of Hb H and/or Bart's even more reliable."

Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in multi-ethnic populations

Van Delft P, Lenters E, Bakker-Verweij M, de Korte M, Baylan U, Harteveld CL, Giordano PC.
Ref. #1, page 40.

"Diagnosis of the high HbA2 β-thal carrier is obtained without problem on the CE apparatus."

Table 2. Showing the number of cases diagnosed as β-thalassaemia trait

Apparatus	Elevated HbA_2, detected	β-thal DNA confirmed	Sensitivity (%)	Overall average HbA_2 ± SD
VARIANT II™	57/57	57	100	5.21 ± 0.58
Capillarys	57/57	57	100	5.38 ± 0.66
HA 8160	51/51	51	100	5.40 ± 0.63
G7	56/56	56	100	6.32 ± 1.52
Ultra[2]	23/23	23	100	4.73 ± 0.73

Multicenter validation of fully automated capillary electrophoresis method for diagnosis of thalassemias and hemoglobinopathies in Thailand

Sangkitporn S, Sangkitporn SK, Tanjatham S, Suwannakan B, Rithapirom S, Yodtup C, Yowang A, Duangruang S.
Ref. #13, page 41.

"The genotypes of all thalassemia subjects (n= 123) in this study were correctly identified by Capillarys 2 System in comparison with the two other chromatographic methods (Table 2)."

Group	N	Hb pattern	Interpretation	Degree of agreement
1	116	A_2A	Normal Hb typing	100%
2	66	A_2A	β-thalassemia carrier	100%
3	2	A_2FA	β-thalassemia homozygote	
4	120	EA	Hb E carrier	100%
5	30	EF	β-thalassemia/ Hb E	100%
6	25	A_2ABart'sH	Hb H disease	100%
7	50	EE	Hb E homozygote	100%
8	1	Abnormal Hb	Hb J Bangkok carrier	100%
9	1	Abnormal Hb	Hb G Makassar carrier	100%
10	1	Abnormal Hb	Hb C carrier	100%

Table 2. Capillarys 2 System results obtained from blood samples of thalassemias and hemoglobinopathies frequently observed in Thailand. Degree of agreement represents percent agreement between final interpretation of Capillarys 2 System and those of HPLC and LPLC techniques used as comparative methods.

PRECISION

CAPILLARYS 2

Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies

Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R.
Ref. #12, page 41.

"The between-run consistency of the CE was evaluated on 17 consecutive runs of the standard preparation containing HbA, HbF, HbS, and HbC. The mean, SD, and coefficient of variation (CV) were as follows: HbA, 28.1% (SD, 0.54%; CV, 1.91%); HbF, 31.0% (SD, 0.43%; CV, 1.39%); HbS, 30.8% (SD, 0.49%; CV, 1.60%); and HbC, 10.2% (SD, 0.28%; CV, 2.76%)."

CAPILLARYS 2 FLEX PIERCING

Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies

Greene DN, Pyle AL, Chang JS, Hoke C and Lorey T.
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Table 1
Interassay variability observed for the most common hemoglobin species using the Capillarys Flex CE. QC 1, 2, and 3 denote different levels and manufacturers of quality material, as described in Materials and methods section.

	Hb A QC 1	Hb A QC 2	Hb A QC 3	Hb A2 QC 1	Hb A2 QC 2	Hb A2 QC 3	Hb F QC 1	Hb F QC 2	Hb S QC 1
Mean (%)	97.4	96.8	55.3	2.6	2.3	4.9	0.97	9.9	29.9
SD	0.07	0.12	0.5	0.07	0.08	0.17	0.07	0.18	0.37
CV (%)	0.1	0.1	0.9	2.6	3.4	3.5	7.1	1.8	1.2

CAPILLARYS 2

Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies

Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R.
Ref. #12, page 41.

"In 228 samples lacking a variant hemoglobin, there was good agreement between the techniques for HbA and HbF. HbA gave a mean value of 96.2% (SD, 5.7%) by CE and 96.8% (SD, 5.5%) by HPLC.."

CAPILLARYS 2 FLEX PIERCING

Integration of Capillarys 2 Flex Piercing (Sebia) in the daily practice of a specialized pathology laboratory

Guis L, Chaumiera A, Le Galla V, Havreza S.
Ref. #20, page 42.

"The correlations between the two techniques for the calculation of the HbA and HbF fractions were excellent, the slopes of the regression line were close to 1 with regression coefficients higher than 0,9."

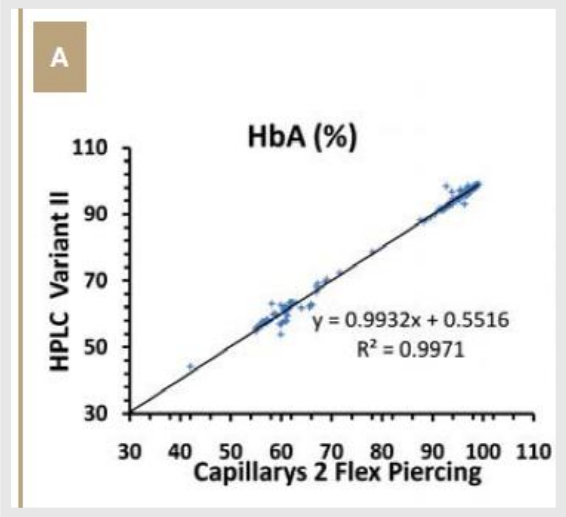


Figure 4 – Correlation of the different hemoglobin fractions between the Capillarys 2 Flex Piercing and the HPLC (Variant II Bio-Rad).

CAPILLARYS 2

Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in multi-ethnic populations

Van Delft P, Lenters E, Bakker-Verweij M, de Korte M, Baylan U, Harteveld CL, Giordano PC.
Ref. #1, page 40.

"The measurement of the fractions is usually very accurate, and since on CE glycated fractions are not separated from HbA, the HbF and HbA2 estimation is not disturbed by overlapping."

Rapid diagnosis of thalassemias and other hemoglobinopathies by capillary electrophoresis system

Winichagoon P, Svasti S, Munkongdee T, Chaiya W, Boonmongkol P, Chantrakul N, Fucharoen S.
Ref. #14, page 41.

"The within-run precision study results showed low variability in the measurement of Hb A2 and Hb F concentrations within the reference range in normal subjects, %CV = 2.06 for Hb A2 and 9.33 for Hb F. The results also showed little within-run variability in the measurement of elevated Hb concentration in the β-thalassemia/Hb E sample, CV = 3.23 for Hb A2, 0.73 for Hb E, and 5.93 for Hb F."

"The measured amounts of Hb A2 were as follows: mean, 2.8%, CV, 2%; mean, 1.5%, CV, 5%; mean, 1.1%, CV, 6%; and mean, 0.5%, CV, 13%. The value of 1.1% was thus considered as the quantification limit for Hb A2."

Interlaboratory comparison of current high-performance methods for HbA2

Paleari R, Gulbis B, Cotton F, Mosca A.
Ref. #2, page 40.

"With regard to the imprecision, the data obtained in our study proven that all methods were performing better than 4.5% CV, as also recently reported (Anagnostopoulos et al., 2009), thus confirming that the quality of the automated HPLC and CE methods is higher with respect to the imprecision obtainable years ago with the minicolumns methods (Brosius et al., 1978), or by cellulose acetate electrophoresis followed by densitometry, or after elution of the HbA and HbA2 bands and spectrophotometric quantitation (International Committee for Standardization in Haemathology 1978)."

"The overall imprecision was between 0.5% and 4.4% (as CV), and no substantial differences in reproducibility were found in relation to the analytical principle of the method (CE or HPLC)."

PRECISION

Multicenter validation of fully automated capillary electrophoresis method for diagnosis of thalassemias and hemoglobinopathies in Thailand

Sangkitporn S, Sangkitporn SK, Tanjatham S, Suwannakan B, Rithapirom S, Yodtup C, Yowang A, Duangruang S.
Ref. #13, page 41.

"Results of inter-laboratory studies demonstrated that all blood samples were clearly differentiated by all laboratories (Table 1). Overall %CV of Hb A2 quantitation was 1.80-2.86%, 1.26-5.13% and 1.08-6.66% for within run, between run and inter-laboratory comparison, respectively."

Precision level	Hb A_2 Percent Hb A_2	Hb A_2 Percent CV
Within run precision		
Quality control material		
Normal Hb A_2 control	2.24-2.56	1.85-2.86
AFSC control	2.44-2.56	1.80-2.24
Between run precision		
Quality control material		
Normal Hb A_2 control	2.45-2.53	2.21-4.11
AFSC control	2.44-2.56	2.13-3.11
EDTA blood sample		
Normal subject	2.81	2.28
β-thalassemia carrier	5.60	1.26
Hb E carrier	3.50	2.02
β-thalassemia/ Hb E	5.25	1.90
β-thalassemia homozygote	2.92	5.13
Inter-laboratory precision		
EDTA blood sample		
Normal subject	2.7	2.18
β-thalassemia carrier	5.34	1.08
Hb E carrier	3.47	6.66
Hb E carrier	3.60	2.78

Table 1. Precision of Hb A2, Hb F and Hb E quantification using Capillarys 2 system

CAPILLARYS 2 FLEX PIERCING

Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies

Greene DN, Pyle AL, Chang JS, Hoke C and Lorey T.
Ref. #5, page 40.

Table 1
Interassay variability observed for the most common hemoglobin species using the Capillarys Flex CE. QC 1, 2, and 3 denote different levels and manufacturers of quality material, as described in Materials and methods section.

	Hb A QC 1	Hb A QC 2	Hb A QC 3	Hb A2 QC 1	Hb A2 QC 2	Hb A2 QC 3	Hb F QC 1	Hb F QC 2	Hb S QC 1
Mean (%)	97.4	96.8	55.3	2.6	2.3	4.9	0.97	9.9	29.9
SD	0.07	0.12	0.5	0.07	0.08	0.17	0.07	0.18	0.37
CV (%)	0.1	0.1	0.9	2.6	3.4	3.5	7.1	1.8	1.2

PRECISION

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

Altinier S, Varagnolo M, Zaninotto M and Plebani M.
Ref. #15, page 41.

"The within run imprecision study in HbA 2 quantification, using control material (mean value 2.31 %) provided a CV = 1.25 % . Between run imprecision study, carried out on the same control and on two patient samples (mean values 2.03 % and 3.73 % , respectively), yielded CV % of 1.52, 3.9 and 3.28, respectively."

Analytical evaluation of the Capillarys 2 Flex piercing for routine haemoglobinopathies diagnosis

Agouti I, Merono F, Bonello-Palot N, Badens C.
Ref. #16, page 41.

"Between-run CVs for both normal and raised HbA2 levels was always found below 2.5% and within-run CVs below 2.13%."

Table 1. Analytical imprecision

	Within run		Between run	
	Mean (%)	CV (%)	Mean (%)	CV (%)
HbA2 Normal	2.32	1.8	2.21	1.43
HbF < 1%	0.59	10.91	0.43	11.23
HbA2 beta-thal carrier	5.70	1.33	4.94	1.05
HbF > 1%	6.38	2.01	2.67	3.08

LINEARITY

CAPILLARYS 2 FLEX PIERCING

Analytical evaluation of the Capillarys 2 Flex piercing for routine haemoglobinopathies diagnosis

Agouti I, Merono F, Bonello-Palot N, Badens C.
Ref. #16, page 41.

"A good linearity was observed for both HbA2 (2.2-6.2%) and HbF (0.6-6.5%) measurements."

"HbA2 value on Capillarys 2 Flex Piercing was identical whatever concentrations tested and hence was not influenced by the haemoglobin concentration (tested range, from 138 to 13.3 g/L, data not shown)."

CAPILLARYS 2

Interlaboratory comparison of current high-performance methods for HbA2

Paleari R, Gulbis B, Cotton F, Mosca A.
Ref. #2, page 40.

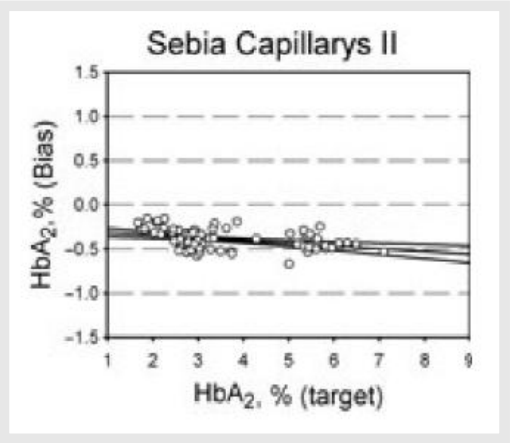


Figure1. Bland–Altman plots. Bias corresponds to the difference between HbA2 results obtained with the tested method and the consensus value (mean of all methods).

Table 3. Linear regression parameters and correlation coefficients calculated from method comparison for HbA_2 assay (x-axis: mean HbA_2 of all methods; n = 80*)

Method	Slope (C.I.)	Intercept (C.I.)	r^2
Bio-Rad Variant I	0.799 (0.767–0.829)	0.57 (0.45–0.68)	0.9736
Bio-Rad Variant II	0.944 (0.922–0.965)	0.34 (0.26–0.42)	0.9900
Menarini HA-8160	0.789 (0.766–0.813)	0.78 (0.69–0.87)	0.9828
Tosoh G7	1.172 (1.149–1.195)	−0.57 (−0.66 to −0.49)	0.9924
Tosoh G8	1.194 (1.179–1.209)	−0.69 (−0.75 to −0.63)	0.9970
Beckman Coulter MDQ	1.094 (1.069–1.120)	−0.17 (−0.27 to −0.08)	0.9894
Beckman Coulter PA800	1.065 (1.047–1.084)	−0.07 (−0.14 to −0.00)	0.9941
Sebia Capillarys II	0.942 (0.925–0.959)	−0.19 (−0.25 to −0.12)	0.9939

*n = 78 for Sebia Capillarys II.

Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies

Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R.
Ref. #12, page 41.

"However, these samples consistently had higher HbA2 percentages by CE (mean, 2.8%; SD, 0.8%) than by HPLC (mean, 2.3%; SD, 0.8%). With the exception of a couple of outliers, this was true at all levels of HbA2. The slope was 0.931 (0.908-0.953) with an intercept of –0.32 (95% confidence interval, –0.38 to –0.25). The correlation coefficient was 0.9832 with a bias of –0.51."*

** "228 samples lacking a variant hemoglobin"*

CORRELATION

Multicenter validation of fully automated capillary electrophoresis method for diagnosis of thalassemias and hemoglobinopathies in Thailand

Sangkitporn S, Sangkitporn SK, Tanjatham S, Suwannakan B, Rithapirom S, Yodtup C, Yowang A, Duangruang S.
Ref. #13, page 41.

"Comparisons of the 3 methods performed to estimate accuracy of percent Hb A2 and percent Hb F showed good linear correlation."

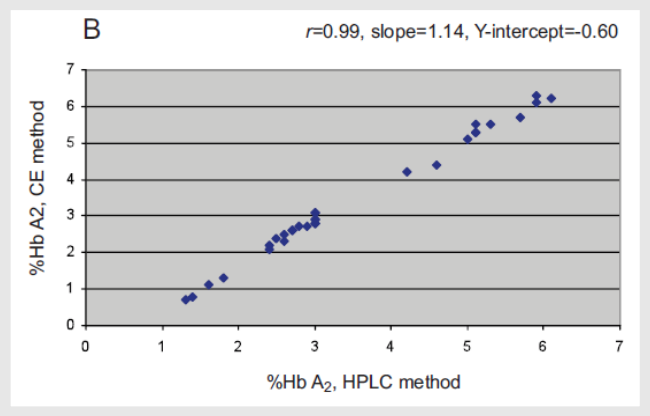


Fig 1 – Comparison of Hb A2 levels between CE and HPLC or LPLC methods at four reference laboratories in Thailand.

CAPILLARYS 2 FLEX PIERCING

Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies

Greene DN, Pyle AL, Chang JS, Hoke C and Lorey T.
Ref. #5, page 40.

"Interestingly, in the patient population without Hb E, S, or C, CE shows a negative bias at the low end (for values ≤3.1% bias=−0.15) and a positive bias at the high end (for values >3.1% bias=0.28), relative to HPLC (Fig. 2D). This bias should allow for CE to obtain a more distinct separation of patients with and without β thalassemia."

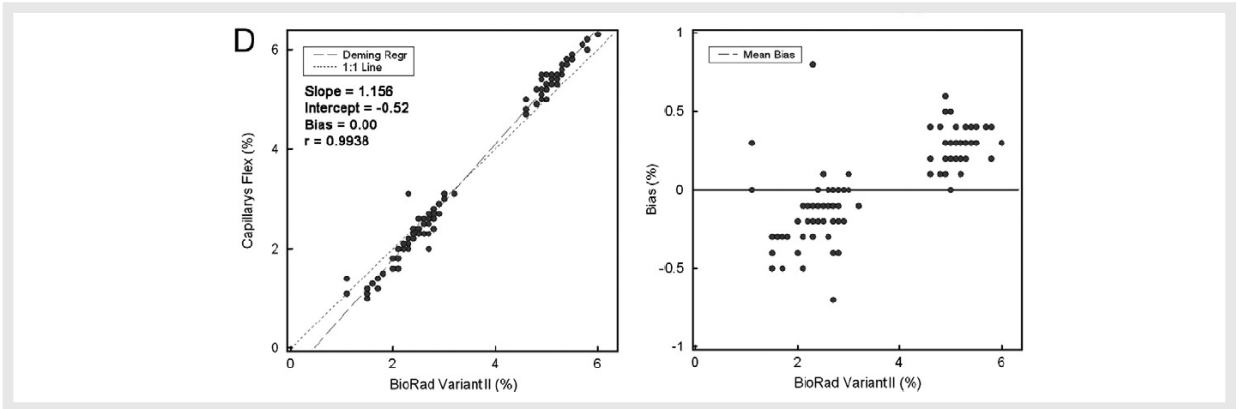


Fig 2. Hb A2 quantitation comparison between HPLC and CE Hb A2 quantified for 167 samples

Integration of Capillarys 2 Flex Piercing (Sebia) in the daily practice of a specialized pathology laboratory

Guis L, Chaumiera A, Le Galla V, Havreza S.
Ref. #20, page 42.

"The correlations between the two techniques for the calculation of the HbA and HbF fractions were excellent, the slopes of the regression line were close to 1 with regression coefficients higher than 0,9. The same result was observed for the Hb A2 quantification."

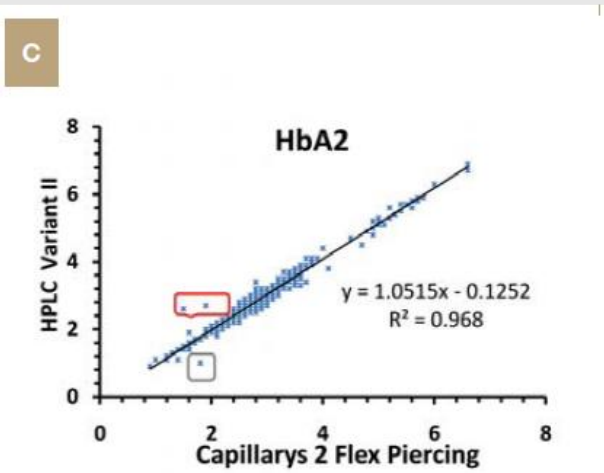


Figure 4 – Correlation of the different hemoglobin fractions between the Capillarys 2 Flex Piercing and the HPLC (Variant II Bio-Rad).

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

Altinier S, Varagnolo M, Zaninotto M and Plebani M.
Ref. #15, page 41.

"The comparison between HbA 2 values obtained using HPLC and those made using CFP was carried out on 392 out of 451 samples yielding the following results at regression analysis: CFP = 1.24×HPLC – 0.64 (slope 95 % CI: from 1.14 to 1.29, intercept 95 % CI: from – 0.81 to – 0.44, r = 0.94) as shown in Figure 1 A. Figure 1B shows the regression analysis of HbA 2 concentrations in the remaining 59 samples, whose pattern included HbS variant: CFP = 0.71×HPLC– 0.27 (slope 95 % CI: from 0.56 to 0.89 intercept 95 % CI: from – 0.36 to 0.84, r = 0.81)."

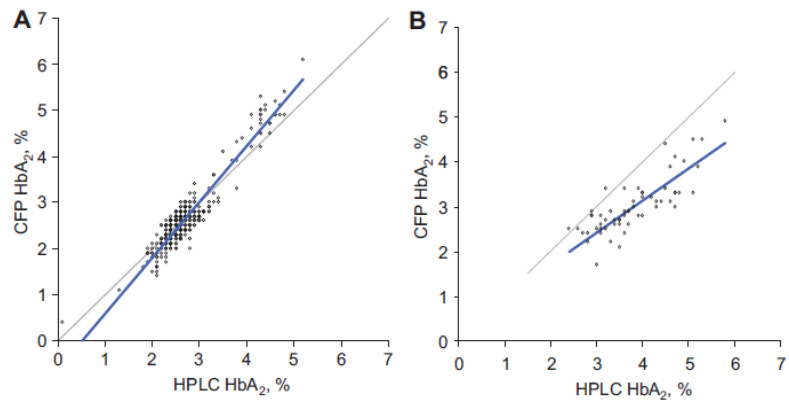


Figure 1 Passing and Bablok regression of HbA_2 measurements obtained in Capillarys 2 flex piercing vs. HPLC.
(A) Samples without any abnormal Hb fraction (n=392): CFP=1.21×HPLC−0.64; (B) samples with HbS variant (n=59): CFP=0.71×HPLC−0.27.

CORRELATION

Analytical evaluation of the Capillarys 2 Flex piercing for routine haemoglobinopathies diagnosis

Agouti I, Merono F, Bonello-Palot N, Badens C.
Ref. #16, page 41.

"A linear correlation for HbA2, HbF and HbS measurement was obtained between the two methods (r = 0.967, 1.00 and 0.998, respectively). The correlation for Hb A2 measurement in the case of the presence of HbS was also evaluated and was found in the same range when compared to HbA2 correlation for all tested samples (r = 0.933 vs. 0.967)."

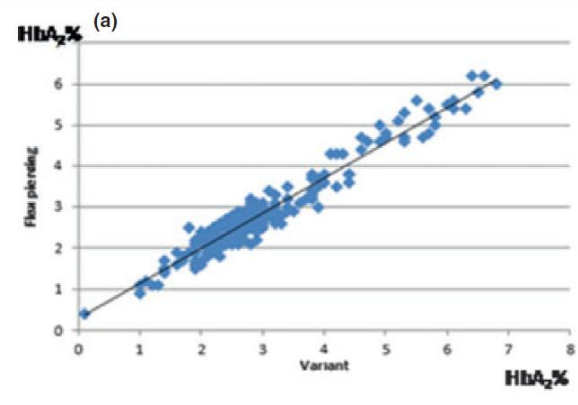


Figure 1. Regression analysis of HbA2 and HbF measurement on the Capillarys 2 Flex Piercing and the Bio-Rad Variant II HPLC system

CAPILLARYS 2

Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in multi-ethnic populations

Van Delft P, Lenters E, Bakker-Verweij M, de Korte M, Baylan U, Harteveld CL, Giordano PC.
Ref. #1, page 40.

"The measurement of the fractions is usually very accurate, and since on CE glycated fractions are not separated from HbA, the HbF and HbA2 estimation is not disturbed by overlapping."

Table 4. Results from samples with HbF range between <1 and 100%, measured by the five machines in a total of 167 runs

	VARIANT II™	Capillarys	HA 8160	G7	*Ultra*²
HbF ± 1% a	1.2	0.8	1.2	1.7	0.7
HbF ± 1% b	2.0	1.1	1.9	2.7	1.2
HbF ± 2% b	3.0	2.2	3.1	3.1	1.9
HbF ± 5%	5.6	4.9	5.5	5.4	4.5
HbF ± 13%	13.3	12.8	11.9	11.7	11.5
Minimum reading	0.10	0	0.10	0.40	0
Maximum reading	110	93.20	68.30	74	79.00

HbF %b and %a represent levels with or without overlapping with the HbA_{1c} peak, respectively.

Interlaboratory comparison of current high-performance methods for HbA2

Paleari R, Gulbis B, Cotton F, Mosca A.
Ref. #2, page 40.

Table 2. Reproducibility of HbF measurement evaluated on samples with HbF higher than 1.5%

Method	Instrumentation	HbF, % Mean	HbF, % SD	CV %	CV *n*
HPLC	Bio-Rad Variant I – β-thal short program	3.2	0.23	7.2	6
	Bio-Rad Variant II – Dual kit	2.5	0.03	1.2	17
	Menarini HA8160	2.6	0.04	1.7	10
	Tosoh G7	2.4	0.14	5.5	16
	Tosoh G8	2.6	0.07	2.6	12
Capillary electrophoresis	Beckman Coulter PA800 – Analis kit	3.0	0.24	8.2	11
	Sebia Capillarys II	2.9	0.08	2.6	5

Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies

Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R.
Ref. #12, page 41.

"The between-run consistency of the CE was evaluated on 17 consecutive runs of the standard preparation contain-ing HbA, HbF, HbS, and HbC. The mean, SD, and coefficient of variation (CV) were as follows: HbA, 28.1% (SD, 0.54%; CV, 1.91%); HbF, 31.0% (SD, 0.43%; CV, 1.39%); HbS, 30.8% (SD, 0.49%; CV, 1.60%); and HbC, 10.2% (SD, 0.28%; CV, 2.76%)."

Multicenter validation of fully automated capillary electrophoresis method for diagnosis of thalassemias and hemoglobinopathies in Thailand

Sangkitporn S, Sangkitporn SK, Tanjatham S, Suwannakan B, Rithapirom S, Yodtup C, Yowang A, Duangruang S.
Ref. #13, page 41.

Precision level	Hb F	
	Percent Hb F	Percent CV
Within run precision		
Quality control material		
Normal Hb A_2 control	-	-
AFSC control	21.7-22.35	0.71-1.86
Between run precision		
Quality control material		
Normal Hb A_2 control	-	-
AFSC control	21.44-21.74	3.59-4.86
EDTA blood sample		
Normal subject	0.32	9.97
β-thalassemia carrier	-	-
Hb E carrier	-	-
β-thalassemia/ Hb E	20.38	2.02
β-thalassemia homozygote	46.05	5.35
Inter-laboratory precision		
EDTA blood sample		
Normal subject	-	-
β-thalassemia carrier	-	-
Hb E carrier	1.5	6.42
Hb E carrier	-	-

Table 1. Precision of Hb A2, Hb F and Hb E quantification using Capillarys 2 system

Rapid diagnosis of thalassemias and other hemoglobinopathies by capillary electrophoresis system

Winichagoon P, Svasti S, Munkongdee T, Chaiya W, Boonmongkol P, Chantrakul N, Fucharoen S.
Ref. #14, page 41.

"The within-run precision study results showed low variability in the measurement of Hb A2 and Hb F concentrations within the reference range in normal subjects, %CV = 2.06 for Hb A2 and 9.33 for Hb F. The results also showed little within-run variability in the measurement of elevated Hb concentration in the β-thalassemia/Hb E sample, CV = 3.23 for Hb A2, 0.73 for Hb E, and 5.93 for Hb F."

PRECISION

CAPILLARYS 2 FLEX PIERCING

Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies

Greene DN, Pyle AL, Chang JS, Hoke C and Lorey T.
Ref. #5, page 40.

Table 1
Interassay variability observed for the most common hemoglobin species using the Capillarys Flex CE. QC 1, 2, and 3 denote different levels and manufacturers of quality material, as described in Materials and methods section.

	Hb A QC 1	Hb A QC 2	Hb A QC 3	Hb A2 QC 1	Hb A2 QC 2	Hb A2 QC 3	Hb F QC 1	Hb F QC 2	Hb S QC 1
Mean (%)	97.4	96.8	55.3	2.6	2.3	4.9	0.97	9.9	29.9
SD	0.07	0.12	0.5	0.07	0.08	0.17	0.07	0.18	0.37
CV (%)	0.1	0.1	0.9	2.6	3.4	3.5	7.1	1.8	1.2

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

Altinier S, Varagnolo M, Zaninotto M and Plebani M.
Ref. #15, page 41.

"In a patient sample with a mean HbF of 1.45 % , the system exhibited a CV % of 3.78."

Analytical evaluation of the Capillarys 2 Flex piercing for routine haemoglobinopathies diagnosis

Agouti I, Merono F, Bonello-Palot N, Badens C.
Ref. #16, page 41.

Table 1. Analytical imprecision

	Within run		Between run	
	Mean (%)	CV (%)	Mean (%)	CV (%)
HbA2 Normal	2.32	1.8	2.21	1.43
HbF < 1%	0.59	10.91	0.43	11.23
HbA2 beta-thal carrier	5.70	1.33	4.94	1.05
HbF > 1%	6.38	2.01	2.67	3.08

RESOLUTION /VARIANTS DETECTION

HbA2 levels in normal, beta-thalassaemia and haemoglobin E carriers by capillary electrophoresis

Hafiza A, Malisa MY, Khirotdin RD, Azlin I, Azma Z, Thong MC, Ali IM, Yeoh ZN, Mohd Ishak L, Mohd Radzi NR, Hussin NH.
Ref. #19, page 42.

"Our study showed a significantly lower HbF level by CE than that of HPLC measured from the normal population. This could be due to the presence of HbA1c fractions that could overlap with HbF in HPLC analysis. […] This was because the glycated HbA fractions were not separated from HbA in CE, thus circumventing the problem of overlap with other haemoglobins."

Analytical evaluation of the Capillarys 2 Flex piercing for routine haemoglobinopathies diagnosis

Agouti I, Merono F, Bonello-Palot N, Badens C.
Ref. #16, page 41.

"In samples with known Hb variants (HbS, HbC, HbD), no interference with HbA2 or HbF estimation was observed."

LINEARITY

CAPILLARYS 2 FLEX PIERCING

Analytical evaluation of the Capillarys 2 Flex piercing for routine haemoglobinopathies diagnosis

Agouti I, Merono F, Bonello-Palot N, Badens C.
Ref. #16, page 41.

"A good linearity was observed for both HbA2 (2.2-6.2%) and HbF (0.6-6.5%) measurements."

CAPILLARYS 2

Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies

Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R.
Ref. #12, page 41.

"In 228 samples lacking a variant hemoglobin, there was good agreement between the techniques for HbA and HbF. (…). HbF gave an identical mean of 0.9% by both techniques with an SD of 5.6% by CE and 5.4% by HPLC."

CAPILLARYS 2 FLEX PIERCING

Analytical evaluation of the Capillarys 2 Flex piercing for routine haemoglobinopathies diagnosis

Agouti I, Merono F, Bonello-Palot N, Badens C.
Ref. #16, page 41.

"A linear correlation for HbA2, HbF and HbS measurement was obtained between the two methods (r = 0.967, 1.00 and 0.998, respectively). It must be noted that the correlation on HbF is better for higher HbF values because the HbF fraction may elute partially overlapping the first HbA1c peak on the Bio-Rad Variant II HPLC. The biological interpretation was the same whatever the method used."

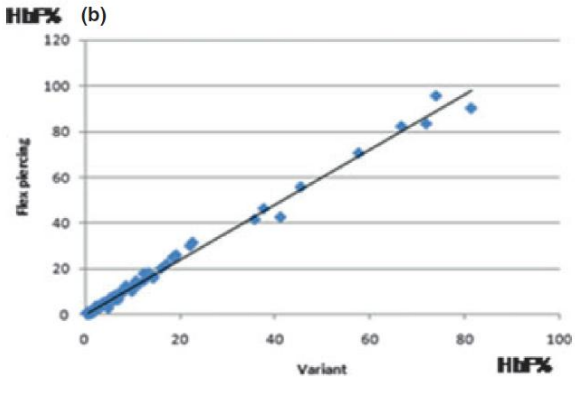


Figure 1. Regression analysis of HbA2 and HbF measurement on the Capillarys 2 Flex Piercing and the Bio-Rad Variant II HPLC system.

Integration of Capillarys 2 Flex Piercing (Sebia) in the daily practice of a specialized pathology laboratory

Guis L, Chaumiera A, Le Galla V, Havreza S.
Ref. #20, page 42.

"The correlations between the two techniques for the calculation of the HbA and HbF fractions were excellent, the slopes of the regression line were close to 1 with regression coefficients higher than 0,9."

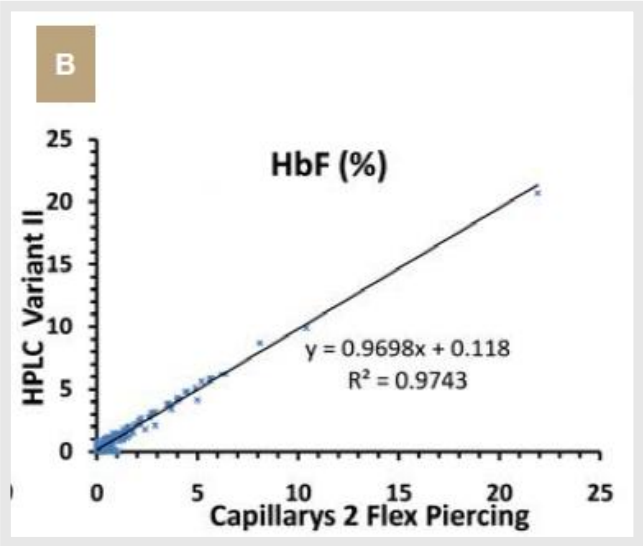


Figure 4 – Correlation of the different hemoglobin fractions between the Capillarys 2 Flex Piercing and the HPLC (Variant II Bio-Rad).

Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies

Greene DN, Pyle AL, Chang JS, Hoke C and Lorey T.
Ref. #5, page 40.

"When all 164 samples were included, the agreement of Hb F was also very good, 1.9% (SD, 4.3%) and 1.7% (SD, 5.3%) for HPLC and CE, respectively (bias=−0.17; r=0.994)."

Comparison of two methods for the quantification and identification of hemoglobin variants

Higgins T, Mack M, Khajuria A.
Ref. #3, page 40.

"The hemoglobin variant identification system on the Sebia Capillarys 2 correctly identified both common and some unusual hemoglobin variants. This is an advantage over using the HPLC with electrophoresis at alkaline and pH since only a single analytical system is used instead of three."

Expression of hemoglobin variant migration by capillary electrophoresis relative to hemoglobin A2 improves precision

Keren DF, Shalhoub R, Gulbranson R, Hedstrom D.
Ref. #10, page 41.

"Developed in the past decade, the Sebia Capillarys has a superior throughput compared with HPLC systems, provides a more straightforward pattern for interpretation, and does not have the need to account for glycated and breakdown products when measuring the most common variant Hbs."

Multicenter validation of fully automated capillary electrophoresis method for diagnosis of thalassemias and hemoglobinopathies in Thailand

Sangkitporn S, Sangkitporn SK, Tanjatham S, Suwannakan B, Rithapirom S, Yodtup C, Yowang A, Duangruang S.
Ref. #13, page 41.

"The Capillarys 2 System (Boonkant et al, 2008; Keren et al, 2008) has been developed for diagnosis of these genetic diseases to provide a rapid and fully automated system, with a throughput of 34 samples per hour. Other advantages of the system include the ability to separate and quantitate Hb A2, Hb H and Hb Bart's, competitive cost and minimal sample manipulation."

Prevention of Thalassaemias and Other Haemoglobin Disorders: Volume 2: Laboratory Protocols

Old J, Harteveld CL, Traeger-Synodinos J, Petrou M, Angastiniotis M, Galanello R.
Ref. #6, page 40.

"Capillary Electrophoresis - accurate in the absence of variants (see above) and high-throughput. Has an advantage over HPLC in that it separates HbE from HbA2, thus providing a clean measurement of HbA2 in patients with HbE."

Advances in detection of hemoglobinopathies

Greene DN, Vaughn CP, Crews BO and Agarwal AM.
Ref. #8, page 40.

"Visually, CZE electropherograms for hemoglobin analysis are much cleaner. HPLC chromatograms tend to reveal multiple post-translational modification and degradation peaks that complicate interpretation. A practical advantage of CZE is that the commercially available CZE instruments have unparalleled software that allows for enhanced interface design and implementation."

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

Altinier S, Varagnolo M, Zaninotto M and Plebani M.
Ref. #15, page 41.

"From an organizational view-point, to obtain the screening of 15 samples, the HPLC system (for quantification) followed by agarose electrophoresis in alkaline pH (to detect hemoglobin variants) calls for more than 2 h of analytical procedure, and some minutes for recording, numbering and hemolysing samples. Alternatively, on using CFP, which provides the detection and quantification of hemoglobins the analytical time falls to about 40 min, and no manual steps are required."

"CFP allows the accurate quantification and identification of physiological hemoglobins and the more common variants, and can therefore replace HPLC and alkaline electrophoresis; thanks to its characteristics, it saves on both time and personnel. Moreover, the possibility of directly using primary tubes significantly decreases the number of the manual steps, known to incur the risk of error. By adopting CFP in routine setting the manual steps are limited to acidic gel electrophoresis, which is required in a limited number of samples."

Analytical evaluation of the Capillarys 2 Flex piercing for routine haemoglobinopathies diagnosis

Agouti I, Merono F, Bonello-Palot N, Badens C.
Ref. #16, page 41.

"Based on the proven technology of the original Capillarys instrument, the new system features cap piercing for analysis of whole blood directly from primary capped sample tubes. Once primary sample tubes are loaded onto the system, the sample rack is gently inverted to ensure whole-blood samples remain homogeneous, and accurate results are produced. Positive sample identification ensures full traceability from primary tube to final result. A total of eight simultaneous migrations and a throughput of 37 tests per hour allow production of rapid results."

"Regarding the utilization of systems, the new cap-piercing capability streamlines workflow and maximizes biohazard safety. It offers considerable advantages in terms of precise quantification, savings in time and full automation."

"When using the Capillarys 2 Flex Piercing, all glycated fractions co-migrate with the main corresponding peak and are not separated (Figure 2), providing an accurate HbA2 quantitative estimation and a clear profile, easy to interpret."

CAPILLARYS 2 FLEX PIERCING

Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies

Greene DN, Pyle AL, Chang JS, Hoke C and Lorey T.
Ref. #5, page 40.

"Both the cap piercer and the capillaries were evaluated for carryover. No carryover was detected."

Analytical evaluation of the Capillarys 2 Flex piercing for routine haemoglobinopathies diagnosis

Agouti I, Merono F, Bonello-Palot N, Badens C.
Ref. #16, page 41.

"No clinically relevant carry-over for HbA2 measurement was noted."

Integration of Capillarys 2 Flex Piercing (Sebia) in the daily practice of a specialized pathology laboratory

Guis L, Chaumiera A, Le Galla V, Havreza S.
Ref. #20, page 42.

"Whereas an inter-samples contamination is usually observed on Bio Rad Variant II with samples containing HbC, no contamination has been observed on Capillarys 2 Flex Piercing."

MIGRATION POSITION

CAPILLARYS 2

Expression of hemoglobin variant migration by capillary electrophoresis relative to hemoglobin A2 improves precision

Keren DF, Shalhoub R, Gulbranson R, Hedstrom D.
Ref. #10, page 41.

"By CE, the mean migration position (x-axis value) and SD for HbA2, HbS, HbC, HbG, and HbD were quite reproducible with CVs that ranged from 0.79% to 1.11%.The 2 most closely migrating variants in this study were HbG and HbD. Both migrated in zone 6, with mean migrations of 205 and 208, respectively."

Table 1
Precision of Variant Location by Migration Position

	HbA_2 (n = 193)	HbS (n = 96)	HbC (n = 54)	HbG (n = 24)	HbD (n = 19)
Mean*	245	215	254	205	208
1 SD	2.3	2.3	2.8	1.6	2.0
Coefficient of variation (%)	0.95	1.06	1.11	0.79	0.96

Hb, hemoglobin.
* Mean migration units (range, 1-300).

CAPILLARYS 2 FLEX PIERCING

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

Altinier S, Varagnolo M, Zaninotto M, Plebani M.
Ref. #15, page 41.

"Migration time imprecision form 43 patient samples with HbS % ranging from 23.3 to 88.4 provided CV = 0.43 % (mean value 213.8). In ten HbC subjects, the mean migration time was 251.0 and CV = 0.69 % . Differently, in 13 HbS-C patient samples, the mean migration time was 211.1 with a CV = 1.80 % for the HbS variant, while the mean HbC running time was 250.15 and the CV = 1.47 %. HbE exhibited in 8 samples a mean migration time of 227.7 and a CV = 0.19 %."

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

Altinier S, Varagnolo M, Zaninotto M and Plebani M.
Ref. #15, page 41.

"The values obtained in our laboratory from four exercises of the " HbF/HbA 2 and abnormal hemoglobins " scheme compared to the target values from all the participants and to the mean value of the participants using Sebia Capillary Electrophoresis are reported in Table 1."

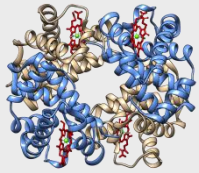
	Obtained value	All methods (mean)	Capillary electrophoresis (mean)
HbA_2%	2.3	2.5	2.4
	2.3	2.4	2.4
	2.6	2.6	2.5
	3.7	3.6	3.7
	2.8	2.9	2.8
	2.5	2.6	2.5
HbF%	9.9	9.0	9.5
	Undetectable	0.3	Not provided
	Undetectable	0.5	Not provided
	Undetectable	0.4	Not provided
	Undetectable	0.2	Not provided
	6.0	5.8	5.9

Table 1 Results from HbA_2/HbF and abnormal hemoglobin UK NEQAS scheme.

Beta-thalassemias: molecular, epidemiological, diagnostical and clinical aspects

Joly P, Pondarre C, Badens C.
Ref. #21, page 42.

"Numerous external quality assessments have clearly demonstrated the superiority of high performance liquid chromatographic (HPLC) and capillary electrophoresis (CE) techniques on the gel electrophoresis techniques with integration of the different fractions which give reliable results only for fractions greater than 15-20% of the total hemoglobin."



Data below are a compilation of reference values for the hemoglobin fractions, issued from several bibliographic references.
These data are for information only. As indicated in the Package Inserts, it is recommended that each laboratory establish its own threshold values.

Qualitatively and quantitatively normal profiles

Interpretation	n	Hb A (%)	Hb F (%)	Hb A2 (%)	Hb variant (%)	Bibliographic Reference
Normal	116		0.3 ± 0.5	2.8 ± 0.3		**#13, page 41**
Normal	45		0.1 ± 0.2	2.5 ± 0.4		**#14, page 41**
Normal	40			1.9 - 2.9 (range) 2.4 (median)		**#2, page 40**
Normal	207			1.9 - 3.1 (range) 2.49 (mean)		**#25, page 43**
Normal	154		0.03 ± 0.24	2.75 ± 0.26		**#19, page 42**

** n: number of samples used to calculate the reference values*

Alpha-thalassemia

Interpretation	n	Hb A (%)	Hb F (%)	Hb A2 (%)	Hb variant (%)	Bibliographic Reference
Hb H disease	25		0.7 ± 0.3	1.2 ± 0.3	3.0 ± 2.0 (Hb H) 1.0 ± 1.1(Hb Bart's)	**#13, page 41**
Hb H disease	26		0.2 ± 0.3	1.0 ± 0.2	6.7 ± 4.8 (Hb H) 1.1 ± 0.7 (Hb Bart's)	**#14, page 41**
Hb H/Hb Constant Spring	9		1.0 ± 0.6	0.7 ± 0.5	11.3 ± 6.5 (Hb H) 4.2 ± 4.1 (Hb Bart's) 2.6 ± 1.4 (Hb Constant Spring)	**#14, page 41**
α^0-thalassemia trait	36		0.3 ± 0.5	2.3 ± 0.2		**#14, page 41**
α^0-thalassemia trait/Hb E	6		0.5 ± 0.8	4.0 ± 0.3	16.3 ± 0.8 (Hb E)	**#14, page 41**
α^+-thalassemia trait	30		0.2 ± 0.4	2.6 ± 0.3		**#14, page 41**
Hb Bart's/Hb E	5		0.9 ± 0.4	3.7 ± 0.2	11.8 ± 0.7 (Hb E)	**#14, page 41**
Hb Bart's/Hb E /Hb Constant Spring	13		2.0 ± 1.1	2.2 ± 0.2	12.6 ± 0.8 (Hb E)	**#14, page 41**

** n: number of samples used to calculate the reference values*

Beta-thalassemia

Interpretation	n*	Hb A (%)	Hb F (%)	Hb A2 (%)	Hb variant (%)	Bibliographic Reference
β-thalassemia trait	66		1.2 ± 1.0	5.8 ± 0.7		**#13, page 41**
β-thalassemia trait	69		0.9 ± 1.4	5.4 ± 0.5		**#14, page 41**
β-thalassemia trait	57			5.38 ± 0.66		**#1, page 40**
β-thalassemia trait	91			3.5 - 6.6 (range) 5.01 (mean)		**#25, page 43**
β-thalassemia trait	218			5.23 ± 0.63		**#19, page 42**
β-thalassemia /Hb E	30		44.0 ± 18.0	5.5 ± 1.1	49.0 ± 16.0 (Hb E)	**#13, page 41**
β-thalassemia /Hb E	48		36.8 ± 13.3	4.9 ± 1.6	50.3 ± 13.8 (Hb E)	**#14, page 41**

** n: number of samples used to calculate the reference values*

Hb S

Interpretation	n*	Hb A (%)	Hb F (%)	Hb A2 (%)	Hb variant (%)	Bibliographic Reference
Hb S heterozygous	6			2.3 - 4.0 (range) 3.1 (median)		**#2, page 40**
Hb S heterozygous	107			2.2 - 3.9 (range) 3.06 (mean)		**#25, page 43**
Hb S heterozygous	39			3.1 ± 0.8	40.6 ± 18.9 (Hb S)	**#12, page 41**
Hb S heterozygous	24				46.7 ± 19.3 (Hb S)	**#5, page 40**

** n: number of samples used to calculate the reference values*

Hb D-Punjab

Interpretation	n*	Hb A (%)	Hb F (%)	Hb A2 (%)	Hb variant (%)	Bibliographic Reference
Hb D-Punjab heterozygous	27			2.0 - 3.6 (range) 2.76 (mean)		**#25, page 43**

** n: number of samples used to calculate the reference values*

Hb C

Interpretation	n*	Hb A (%)	Hb F (%)	Hb A2 (%)	Hb variant (%)	Bibliographic Reference
Hb C heterozygous	6			2.2 - 3.6 (range) 3.1 (median)		**#2, page 40**
Hb C heterozygous	19			1.6 - 4.1 (range) 2.91 (mean)		**#25, page 43**
Hb C heterozygous	9				35.8 ± 5.75 (Hb C)	**#5, page 40**

** n: number of samples used to calculate the reference values*

Hb E

Interpretation	n*	Hb A (%)	Hb F (%)	Hb A2 (%)	Hb variant (%)	Bibliographic Reference
Hb E heterozygous	120		0.8 ± 1.1	4.0 ± 0.4	24.0 ± 3.0 (Hb E)	**#13, page 41**
Hb E heterozygous	85		0.4 ± 0.8	3.5 ± 0.4	25.6 ± 1.4 (Hb E)	**#14, page 41**
Hb E heterozygous	52			3.4 ± 0.4		**#11, page 41**
Hb E heterozygous	91			3.58 ± 0.44	24.28 ± 3.38	**#19, page 42**
Hb E heterozygous	26			2.8 - 4.5 (range) 3.65 (mean)		**#25, page 43**
Hb E homozygous	56		2.5 ± 3.1	4.1 ± 0.8	92.9 ± 3.3 (Hb E)	**#14, page 41**
Hb E homozygous	7			4.4 ± 0.4		**#11, page 41**
α^0-thalassemia trait/Hb E	6		0.5 ± 0.8	4.0 ± 0.3	16.3 ± 0.8 (Hb E)	**#14, page 41**
Hb Bart's/Hb E	5		0.9 ± 0.4	3.7 ± 0.2	11.8 ± 0.7 (Hb E)	**#14, page 41**
Hb Bart's/Hb E /Hb Constant Spring	13		2.0 ± 1.1	2.2 ± 0.2	12.6 ± 0.8 (Hb E)	**#14, page 41**
β-thalassemia /Hb E	30		44.0 ± 18.0	5.5 ± 1.1	49.0 ± 16.0 (Hb E)	**#13, page 41**
β-thalassemia /Hb E	48		36.8 ± 13.3	4.9 ± 1.6	50.3 ± 13.8 (Hb E)	**#14, page 41**

** n: number of samples used to calculate the reference values*

Hb Constant Spring

Interpretation	n*	Hb A (%)	Hb F (%)	Hb A2 (%)	Hb variant (%)	Bibliographic Reference
Hb Constant Spring heterozygous	21		0.66 ± 0.8	2.1 ± 0.3	0.52 ± 0.52 (Hb Constant Spring)	**#14, page 41**
Hb Constant Spring heterozygous	70				0.6 ± 0.1	**#9, page 41**
Hb Constant Spring homozygous	10		0.8 ± 0.8	1.3 ± 0.6	3.5 ± 2.5 (Hb Constant Spring)	**#14, page 41**
Hb H/Hb Constant Spring	9		1.0 ± 0.6	0.7 ± 0.5	11.3 ± 6.5 (Hb H) 4.2 ± 4.1 (Hb Bart's) 2.6 ± 1.4 (Hb Constant Spring)	**#14, page 41**
Hb Bart's/Hb E /Hb Constant Spring	13		2.0 ± 1.1	2.2 ± 0.2	12.6 ± 0.8 (Hb E)	**#14, page 41**

** n: number of samples used to calculate the reference values*

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Greene DN, Pyle AL, Chang JS, Hoke C, Lorey T.
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#9. **Screening for Hb Constant Spring in the Guangdong Province, South China, using the Sebia capillary electrophoresis system**

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Keren DF, Shalhoub R, Gulbranson R, Hedstrom D.
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#16. **Analytical evaluation of the Capillarys 2 Flex piercing for routine haemoglobinopathies diagnosis**

Agouti I, Merono F, Bonello-Palot N, Badens C.
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Guis L, Chaumier A, Le Gall V, Havrez S.
RFL (Revue Francophone des Laboratoires) Vol. 43 n°449 – 47-56 (2013)

#21. **Beta-thalassemias: molecular, epidemiological, diagnostical and clinical aspects**

Joly P, Pondarre C, Badens C.
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#22. **Detection of Hb Constant Spring by a capillary electrophoresis method**

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#25. **Quantification of HbA(2) in patients with and without beta-thalassemia and in the presence of HbS, HbC, HbE, and HbD Punjab hemoglobin variants: comparison of two systems.**

Higgins TN, Khajuria A, Mack M.
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