

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

k112491

**B. Purpose for Submission:**

New device

**C. Measurand:**

Hemoglobin A, F, A2, S, C, E, D

**D. Type of Test:**

Quantitative, Capillary Electrophoresis

**E. Applicant:**

Sebia, Inc.

**F. Proprietary and Established Names:**

CAPILLARYS HEMOGLOBIN(E) using the CAPILLARYS 2 instrument

**G. Regulatory Information:**

1. Regulation section:  
21 CFR § 864.7415, Abnormal hemoglobin assay
2. Classification:  
Class II
3. Product code:  
GKA, Abnormal Hemoglobin Quantitation
4. Panel:  
Hematology (81)

**H. Intended Use:**

1. Intended use(s):  
The CAPILLARYS HEMOGLOBIN(E) kit is designed for the separation of the normal hemoglobins (A, A2 and F) in human blood samples, and for the detection of the major hemoglobin variants (S, C, E and D), by capillary electrophoresis in alkaline buffer (pH 9.4) with the SEBIA CAPILLARYS 2 instrument. The CAPILLARYS HEMOGLOBIN(E) kit is designed for laboratory use.

SEBIA CAPILLARYS 2 instrument is an automated analyzer which performs a complete hemoglobin profile for the quantitative analysis of the normal hemoglobin fractions A, A2 and F and for the detection of major hemoglobin variants (S, C, E and D). The assay is performed on the hemolysate of packed red blood cells from blood samples collected in

tubes containing K<sub>2</sub>EDTA or K<sub>3</sub>EDTA as anticoagulant.

For In Vitro Diagnostic Use

2. Indication(s) for use:  
Same as Intended Use
3. Special conditions for use statement(s):  
Prescription Use Only
4. Special instrument requirements:  
For use with the SEBIA CAPILLARYS 2 Instrument

**I. Device Description:**

The CAPILLARYS HEMOGLOBIN (E) kit consist of five components; (1) HEMOGLOBIN(E) buffer contains alkaline buffer (pH 9.4), supplied in 700 mL vials, (2) Hemolyzing Solution, supplied in 700 mL vials, (3) Washing Solution, supplied in 75 mL vials, (4) Dilution segments, supplied in pack of 90, (5) Filters, four per kit. The CAPILLARYS HEMOGLOBIN (E) kit is used in conjunction with a SEBIA CAPILLARYS 2 instrument. The hemoglobin, separated in silica capillaries, is directly detected at an absorbance wavelength of 415 nm. The resulting electrophoregrams are evaluated visually for pattern abnormalities. Direct detection provides relative quantification of individual hemoglobin fractions.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
BIO-RAD VARIANT™ II β-thalassemia Short Program
2. Predicate 510(k) number(s):  
k991127

3. Comparison with predicate:

Similarities		
<i>Item</i>	<b><i>CAPILLARYS HEMOGLOBIN(E) with CAPILLARYS 2 INSTRUMENT</i></b>	<b><i>BIO-RAD VARIANT™ II β-thalassemia Short Program</i></b>
Intended use	<p>The CAPILLARYS HEMOGLOBIN (E) kit is designed for the separation of the normal hemoglobins (A, A2 and F) in human blood samples, and for the detection of the major hemoglobin variants (S, C, E and D), by capillary electrophoresis in alkaline buffer (pH 9.4) with the SEBIA CAPILLARYS 2 instrument. The CAPILLARYS HEMOGLOBIN(E) kit is designed for laboratory use.</p> <p>SEBIA CAPILLARYS 2 instrument is an automated analyzer which performs a complete hemoglobin profile for the quantitative analysis of the normal hemoglobin fractions A, A2 and F and for the detection of major hemoglobin variants (S, C, E and D). The assay is performed on the hemolysate of packed red blood cells from blood samples collected in tubes containing K<sub>2</sub>EDTA or K<sub>3</sub>EDTA as anticoagulant.</p> <p>For In Vitro Diagnostic Use.</p>	<p>BIO-RAD VARIANT™ II β-thalassemia Short Program is intended for the separation and area percent determinations of hemoglobins A2 and F as an aid in the identification of abnormal hemoglobins in whole blood using ion-exchange high performance liquid chromatography ( HPLC).</p> <p>VARIANT™ II β-thalassemia Short Program is intended for use only with the Bio-Rad VARIANT II Hemoglobin Testing System.</p> <p>For in vitro diagnostic use.</p>
Sample hemolysis	Performed automatically by the system	Same
Collection tubes	K <sub>2</sub> or K <sub>3</sub> EDTA anticoagulant	EDTA anticoagulant

<i>Differences</i>		
<i>Item</i>	<i>CAPILLARYS HEMOGLOBIN(E) with CAPILLARYS 2 INSTRUMENT</i>	<i>BIO-RAD VARIANT™ II β-thalassemia Short Program</i>
Separation System	Free solution capillary electrophoresis (FSCE): protein separation in an alkaline buffer (pH 9.4) according to their charge to the electrolyte pH and electroosmotic flow. Electrophoregrams show separated fractions according to their charge.	Ion-exchange high performance liquid chromatography (HPLC): protein separation on the column based on their ionic interaction with the cartridge material and elution by buffer gradient with increasing ionic strength. Chromatograms show retention times of eluted fractions.
Number of separation units	7 parallel capillaries (total capillaries on CAPILLARYS 2 instrument is 8). Position #8 is utilized for the hemolysing reagent	1 column
Use of hemolysis solution	Solution poured in a tube and placed on sample rack	Solution in the VARIANT II Sampling Station
Automated sample introduction	Continuous loading with sample racks	Sequential injection
Analysis throughput	33 samples/hour	9-10 samples/hour
Sample tube processing in the instrument	Aspiration of hemolysate of packed red blood cells from uncapped tube	Aspiration of whole blood from closed tube
Kit components	All components provided together (boxed).	All components provided separately.
Control for migration	SEBIA normal Hb A2 Control (required but provided separately from kit)	Hemoglobin A2/F Calibrator/diluent set
Use of other controls	Yes, (SEBIA Pathological Hb A2 Control, Hb AFSC Control and Hb AF Control)	No
Hb variants library (on-board)	Yes, (displayed by software and indicated in the package insert)	No
Sample identification	Barcode reader on sample racks and tubes	Barcode reader on sample tube
Absorbance wavelength	415 nm	415 and 690 nm

**K. Standard/Guidance Document Referenced (if applicable):**

EP9-A2 *Method Comparison and Bias Estimation Using Patient Samples*, Approved Standard-Second Edition, CLSI

EP05-A2 *Evaluation of Precision Performance of Clinical Chemistry Device Approved Guideline*, CLSI

EP06-A *Evaluation of the Linearity of Quantitative Measurement Procedures*, Approved Guideline, CLSI

EP7-P *Interference Testing in Clinical Chemistry*, CLSI

FDA Use of Symbols on Labels and in Labeling of In-vitro Diagnostic Devices Intended for Professional Use, November 2004

FDA Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices - Guidance for Industry and FDA Staff, May 11, 2005

**L. Test Principle:**

The CAPILLARYS HEMOGLOBIN(E) using the CAPILLARYS 2 instrument uses the principle of capillary electrophoresis in a free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific pH. Separation also occurs according to the electrolyte pH and electroosmotic flow. The CAPILLARYS 2 instrument has capillaries functioning in parallel allowing seven simultaneous analyses for hemoglobin quantification. A sample dilution with hemolysing solution is prepared and injected by aspiration at the anodic end of the capillary. A high voltage protein separation is then performed and direct detection of the hemoglobins is made at 415 nm at the cathodic end of the capillary. The hemoglobins, separated in silica capillaries, are directly and specifically detected at an absorbance wavelength of 415 nm which is specific to hemoglobins. The Hb fractions are separated by absorbance spectrophotometry and identified automatically by the instrument software. The results are expressed as the corrected area percentage for each Hb fraction. The resulting electrophoregrams are evaluated visually for pattern abnormalities. By using alkaline pH buffer, normal and abnormal (or variant) hemoglobins are detected.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

Reproducibility studies were performed using seven different samples (4 blood samples and 3 controls, normal and pathological, identified in the table below). These samples were analyzed on all capillaries of a single CAPILLARYS 2 instrument with one lot of CAPILLARYS HEMOGLOBIN(E) kits. This study included 40 runs over 20 days (two runs per day). Samples were analyzed in duplicate for each run. A summary of the studies expressed as %CV for hemoglobin A, A2, S and F met the acceptance criteria and is outlined in the following tables:

The tables below represent reproducibility between capillaries on the same instrument.

**HbA reproducibility study summary**

HbA %	Normal blood	Blood sample with elevated HbA2	Blood with low HbA2 level	Pathological blood with HbF & HbS	Normal HbA2 control	Pathological HbA2 control	HbAF control
n=120 Sample #	1	2	3	4	5	6	7
Mean Hb%	97.5	96.1	98.2	49.0	98.3	93.7	33.7
Repeatability (CV%)	0.0	0.0	0.0	0.2	0.0	0.1	0.5
Between-run (CV%)	0.0	0.0	0.0	0.1	0.0	0.1	1.1
Between-day (CV%)	0.0	0.0	0.0	0.1	0.0	0.1	2.1
Total (CV%)	0.0	0.0	0.0	0.1	0.0	0.1	1.1
SD	0.5	0.5	0.5	0.21	0.08	0.10	0.48
95% CI	0.04-0.06	0.04-0.06	0.04-0.06	0.18-0.27	0.07-0.10	0.14-0.22	0.51-0.81

Acceptance criteria: %CV <10%

**HbA2 reproducibility study summary**

HbA2 %	Normal blood	Blood sample with elevated HbA2	Blood with low HbA2 level	Pathological blood with HbF & HbS	Normal HbA2 control	Pathological HbA2 control	HbAF control
n=120 Sample #	1	2	3	4	5	6	7
Mean Hb CV%	2.5	3.9	1.8	2.8	2.7	6.5	/
Repeatability (CV%)	1.5	1.0	2.3	1.5	1.7	0.9	/
Between- run (CV%)	1.0	0.3	1.5	0.0	1.6	0.7	/
Between- day (CV%)	0.0	0.6	0.0	0.4	1.6	0.7	/
Total (CV%)	0.0	0.0	0.0	0.1	0.0	0.1	/
SD	0.05	0.05	0.05	0.05	0.08	0.17	/
95% CI	0.04-0.06	0.05-0.06	0.04-0.06	0.04-0.05	0.07-0.10	0.14-0.22	/

Acceptance criteria: %CV <10%

**HbF reproducibility study summary**

HbF %	Normal blood	Blood sample with elevated HbA2	Blood with low HbA2 level	Pathological blood with HbF & HbS	Normal HbA2 control	Pathological HbA2 control	HbAF control
n=120 Sample #	1	2	3	4	5	6	7
Mean HbF%	/	/	/	12	/	/	65.8
Repeatability (CV%)	/	/	/	0.5	/	/	0.2
Between- run (CV%)	/	/	/	0.0	/	/	0.6
Between- day (CV%)	/	/	/	0.4	/	/	1.0
Total (CV%)	/	/	/	0.6	/	/	1.1
SD				0.19			0.51
95% CI	/	/	/	0.16-0.24	/	/	0.49-0.79

Acceptance criteria: %CV <10%

**HbS study reproducibility summary**

HbS %	Normal blood	Blood sample with elevated HbA2	Blood with low HbA2 level	Pathological blood with HbF & HbS	Normal HbA2 control	Pathological HbA2 control	HbAF control
n=120 Sample #	1	2	3	4	5	6	7
Mean Hb S%	/	/	/	36.2	/	/	/
Repeatability (CV%)	/	/	/	0.2	/	/	/
Between- run (CV%)	/	/	/	0.1	/	/	/
Between- day (CV%)	/	/	/	0.2	/	/	/
Total (%)	/	/	/	0.3	/	/	/
SD	/	/	/	0.19	/	/	/
95% CI	/	/	/	0.16-0.24	/	/	/

Acceptance criteria: % CV <20%

**Reproducibility between-instruments and between-lots:**

This reproducibility study was conducted using 7 different blood samples (identified in table below) that were tested twice a day in duplicate for four days. Results were obtained using the CAPILLARYS HEMOGLOBIN(E) procedure with 3 lots of CAPILLARYS HEMOGLOBIN(E) kits and three CAPILLARYS 2 instruments. Reproducibility values were within acceptance criteria for quantitative analysis for each hemoglobin component (A, A2, F, and S). The following table summarizes the total instrument/lot CV% for the individual hemoglobins A, A2, F and S fractions tested.

**Between-instrument reproducibility study summary for HbA**

HbA %	Normal blood	Blood sample with elevated HbA2	Blood with low HbA2 level	Pathological blood with HbF & HbS	Normal HbA2 control	Pathological HbA2 control	HbAF control
n=48 Sample #	1	2	3	4	5	6	7
Mean Hb A%	97.5	96.1	98.2	49.0	98.3	93.7	33.7
Between-instrument (CV%)	0.0	0.1	0.0	0.2	0.1	0.1	0.6
Total (CV%)	0.0	0.1	0.1	0.4	0.1	0.1	1.9
SD	0.4	0.6	0.5	0.214	0.12	0.12	0.68
95% CI	0.03-0.04	0.04-0.07	0.04-0.06	0.18-0.38	0.10-0.17	0.10-0.17	0.65-1.02

Acceptance criteria: %CV <10%

**Between-instrument reproducibility study summary for HbA2**

HbA2 %	Normal blood	Blood sample with elevated HbA2	Blood with low HbA2 level	Pathological blood with HbF & HbS	Normal HbA2 control	Pathological HbA2 control	HbAF control
n=48 Sample #	1	2	3	4	5	6	7
Mean Hb A2%	97.5	96.1	98.2	49.0	98.3	93.7	/
Between-instrument (CV %)	1.7	0.3	2.3	1.3	2.1	1.2	/
Total (CV %)	2.0	1.4	2.7	1.4	3.0	2.6	/
SD	0.5	0.5	0.5	0.05	0.12	0.10	
95% CI	0.04-0.07	0.04-0.06	0.04-0.07	0.04-0.07	0.10-0.17	0.14-0.22	/

Acceptance criteria: %CV <10%

**Between-instrument reproducibility study summary for HbF**

HbF %	Normal blood	Blood sample with elevated HbA2	Blood with low HbA2 level	Pathological blood with HbF & HbS	Normal HbA2 control	Pathological HbA2 control	HbAF control
n=48 Sample #	1	2	3	4	5	6	7
Mean Hb S%	/	/	/	12	/	/	65.8
Between-instrument (CV%)	/	/	/	0.04	/	/	0.3
Total (%)	/	/	/	0.06	/	/	0.9
SD	/	/	/	0.08	/	/	0.61
95% CI	/	/	/	0.06-0.12	/	/	0.37-1.03

Acceptance criteria: % CV <10%

**Between-reproducibility study summary for HbS**

<b>HbS %</b>	Normal blood	Blood sample with elevated HbA2	Blood with low HbA2 level	Pathological blood with HbF & HbS	Normal HbA2 control	Pathological HbA2 control	HbAF control
n=48 Sample #	1	2	3	4	5	6	7
Mean HbS%	/	/	/	36.2	/	/	/
Between-instrument (CV%)	/	/	/	0.4	/	/	/
Total (%)	/	/	/	0.7	/	/	/
SD	/	/	/	0.13	/	/	/
95% CI	/	/	/	0.10-0.19	/	/	/

Acceptance criteria: %CV <20%

A separate reproducibility study was conducted to demonstrate reproducibility for HbC, HbD and HbE using the CAPILLARYS HEMOGLOBIN(E) assay kit with the CAPILLARYS 2 instrument. Nine different samples (three samples per hemoglobin variant) containing HbC, HbD or HbE were analyzed on three different CAPILLARYS 2 instruments using three different lots of buffer and hemolysing solutions for four days (at two different times per day). Within each run samples were analyzed in duplicate. The quantitative results were analyzed to demonstrate reproducibility for HbC, HbD or HbE fractions (mean and CV). The results are provided below:

**HbC reproducibility study summary**

	<b>Sample C1</b>	<b>Sample C2</b>	<b>Sample C3</b>
<b>HbC %</b> n=48	Pathological Sample with HbC	Pathological Sample with HbC	Pathological Sample with HbC
Mean Hb%	45.2	31.0	33.2
Repeatability (CV%)	0.5	0.7	0.7
Between-run (CV%)	0.6	1.1	0.6
Between-day (CV%)	1.4	1.4	1.2
Total Between-instrument	1.6	1.4	1.5
SD/CV%	0.48/1.5	0.48/1.5	0.48/1.5
95% CI	0.36 – 0.71	0.36 – 0.71	0.36 – 0.71

Acceptance criteria: %CV <10%

**HbD reproducibility study summary**

	<b>Sample D1</b>	<b>Sample D2</b>	<b>Sample D3</b>
<b>HbD %</b> n=48	Pathological Sample with HbD	Pathological Sample with HbD	Pathological Sample with HbD
Mean Hb%	40.9	38.5	36.4
Repeatability (CV%)	0.3	0.3	0.3
Between-run (CV%)	0.2	0.2	0.2
Between-day (CV%)	1.1	1.0	1.0
Total Between-instrument	1.2	1.1	1.1
SD/CV%	0.47/1.2	0.47/1.2	0.47/1.2
95% CI	0.34 – 0.78	0.34 – 0.78	0.34 – 0.78

Acceptance criteria: %CV <10%

**HbE reproducibility study summary**

	<b>Sample E1</b>	<b>Sample E2</b>	<b>Sample E3</b>
<b>HbE %</b> n=48	Pathological Sample with HbE	Pathological Sample with HbE	Pathological Sample with HbE
Mean Hb%	25.5	27.1	22.9
Repeatability (CV%)	0.4	0.6	0.7
Between-run (CV%)	0.6	0.5	1.0
Between-day (CV%)	1.0	0.6	0.8
Total Between-instrument	1.3	0.7	1.5
SD/CV%	0.34/1.5	0.34/1.5	0.34/1.5
95% CI	0.27 – 0.48	0.27 – 0.48	0.27 – 0.48

Acceptance criteria: %CV <10%

The reproducibility results of the CAPILLARYS HEMOGLOBIN(E) assay kit with the CAPILLARYS 2 instrument met acceptance criteria for all hemoglobins tested (HbA, HbA2, HbF, HbS, HbC, HbD and HbE).

**B. Linearity/assay reportable range:**

Mixtures of two blood samples (normal blood with hemoglobin fraction A, A2, S, F and umbilical cord samples) were prepared using serial dilutions of saline and normal blood. A separate linearity study was performed using different pathological blood samples with hemoglobin fractions C, D and E and serially diluted 11 times with normal whole and analyzed using the CAPILLARYS HEMOGLOBIN(E) system. The samples were analyzed using the CAPILLARYS HEMOGLOBIN (E) procedure with the CAPILLARYS 2 system according to the guidelines in CLSI EP6-A. The tests were determined to be linear within the entire range studied for each of the following hemoglobin fractions: HbA: 0 – 97.4%, HbA2: 0 – 9.9 %, HbF: 0 – 87.8 %, HbS: 0 – 71.3%, HbC: 0 – 30%, HbD: 0 – 39.4%, HbE: 0 – 95.4%.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*  
Not applicable
- d. *Detection limit:*  
The reportable ranges for normal hemoglobin are:  
Hemoglobin A (HbA): 96.7 – 97.8%  
Hemoglobin F (HbF): < 0.5%  
Hemoglobin A2 (HbA2): 2.2 – 3.2%  
Major hemoglobin variants (S, C, E and D) should not be present in normal hemoglobin samples.  
See the linearity study above for data on recovery of samples across the measuring range for all hemoglobin fractions.
- e. *Analytical specificity:*  
Interference studies were performed on three different whole blood samples: (1) a normal sample (Hb A and Hb A2), (2) a sample with increased Hb A2 level and (3) a pathological sample (with Hb S) to verify a lack of analytical interference due to the presence of bilirubin or triglyceride. Each sample was analyzed 3 times to detect hemoglobin variants A, A2, F and S for reproducibility. Study results indicate the presence of bilirubin does not interfere in concentrations  $\leq 17.9$  mg/dL, and the presence of triglyceride does not interfere in concentrations  $\leq 22.34$  g/L.
- f. *Assay cut-off:*  
Not applicable

**2. Comparison studies:**

- a. *Method comparison with predicate device:*  
Comparison studies were performed using CAPILLARYS HEMOGLOBIN(E) assay kit with the CAPILLARYS 2 instrument compared to the predicate (BIO-RAD VARIANT™ II  $\beta$ -thalassemia Short Program) at three sites on blood samples with normal and abnormal levels for HbA, HbA2, HbF, HbS and variants HbC, HbD and HbE. The blood samples and their corresponding diagnosis assessments were provided by hospitals and laboratories in France and the United States. A total of 362 (226 normal/136 abnormal) donors and patients samples were analyzed at one internal and two external laboratory sites. The subjects included males and females within the age range of  $\leq 1$  year to adult ( $\geq 21$ ). The CAPILLARYS 2 instrument was not evaluated in the newborn/neonate population. The acceptance criterion was defined as the correlation coefficient ( $r$ )  $> 0.90$ . A regression analysis was calculated, producing a slope, intercept and correlation coefficient ( $r$ ) for each hemoglobin fraction at each study site. Regression analysis is not provided for abnormal hemoglobin variants HbC, HbD and HbE at each site due to the small sample size.

**Site 1** (internal) included 56 different normal or pathological blood samples for the HbA2 fraction with or without hemoglobin variants (36 blood samples without abnormal hemoglobin (or Hb variants) and 20 blood samples with abnormal hemoglobin). Results were as follows:

Hb fraction	n	r <sup>2</sup>	y-intercept	slope	95% CI	Range of values (%)
Hb A	56	0.995	-11.24	1.32	-13.35 to -9.13	20.9 – 98.1
Hb A2	44	0.977	-0.07	1.13	-0.13 to -0.01	0.1 – 6.2
Hb F	56	1.000	-0.35	0.93	-0.45 to -0.25	0.0 – 79.0
Hb S	8	0.997	-0.39	1.07	-1.42 to 0.64	6.5 – 40.9

Acceptance criteria: r<sup>2</sup> = >90%

Note: Additional data and supplemental statistical analyses were requested from the sponsor to assess the high y-intercept value for HbA at one of the method comparison sites and a noted overall higher test results for %HbA in the new device when compared to the predicate. A statistical review of the combined data for HbA at all sites indicated that this variant demonstrated a good fit to the linear model, with R<sup>2</sup> above 0.97 for the new device as compared to the predicate. In addition, the sponsor cited literature to support higher device test results for %HbA in the new device versus the predicate based on the differences between capillary electrophoresis and HPLC technology.

This Site 1 study included samples with 20 abnormal variants. Two (2) HbC, four (4) HbD and four (4) HbE variants were detected and were in agreement with the comparative HPLC system.

**Site 2** (external) included 123 different normal or pathological blood samples for the HbA2 fraction with or without hemoglobin variants (90 blood samples without abnormal hemoglobin (or Hb variants) and 33 blood samples with abnormal hemoglobin). Results were as follows:

Hb fraction	N	r <sup>2</sup>	y-intercept	slope	95% CI	Range of values (%)
Hb A	121	0.999	-1.72	1.14	-1.82 to -1.62	0.0 – 98.8
Hb A2	93	0.983	-0.21	1.12	-0.32 to -0.10	1.0 – 6.6
Hb F	103	0.995	-0.62	0.99	-0.82 to -0.43	0.0 – 90.0
Hb S	26	0.988	1.57	0.99	-0.42 to 3.57	14.7 – 54.9
Hb C	5	0.999	0.42	0.99	-1.87 to 1.04	10.0 – 43.7

Acceptance criteria: r<sup>2</sup> = >90%

This Site 2 study site included samples with 33 abnormal variants. Five (5) HbC and two (2) HbE variants were detected and were in agreement with the comparative HPLC system.

**Site 3** (external) included 183 different normal or pathological blood samples for the HbA2 fraction with or without hemoglobin variants (100 blood samples without abnormal hemoglobin (or Hb variants) and 83 blood samples with abnormal hemoglobin). The results are as follows:

<b>Hb fraction</b>	<b>n</b>	<b>r<sup>2</sup></b>	<b>y-intercept</b>	<b>slope</b>	<b>95% CI</b>	<b>Range of values (%)</b>
Hb A	180	0.991	-1.75	1.13	-1.85 to -1.64	0.0 – 98.2
Hb A2	113	0.925	-0.14	1.02	-0.37 to 0.09	0.4 – 6.0
Hb F	181	0.991	0.13	1.06	-0.17 to -0.11	0.0 – 98.0
Hb S	67	0.964	2.19	0.97	1.94 to 3.05	7.4 – 93.6
Hb C	13	0.985	1.29	0.92	-0.49 to 3.07	12.8 – 45.3

Acceptance criteria:  $r^2 = >90\%$

This Site 3 study included samples with 83 abnormal hemoglobin variants. Thirteen (13) HbC and three (3) HbD variants were detected and were in agreement with the comparative HPLC system.

The combined number of abnormal hemoglobin variants detected for all sites in the comparison study is as follows: HbS = 113, HbC = 20, HbD = 7, HbE = 6.

For all sites, abnormal hemoglobins or abnormal levels of normal hemoglobins detected were in agreement with the comparative HPLC system. There were no observed false positives (i.e., detection of an abnormal band or abnormal level of a normal band where no such abnormality existed).

*b. Matrix comparison:*

A study was conducted to assess the difference in the use of K<sub>2</sub>EDTA and K<sub>3</sub>EDTA. A total of 20 samples (15 normal and 5 abnormal) samples were tested the CAPILLARYS 2 system using 3 lots of CAPILLARYS HEMOGLOBIN(E) assay kits. The same samples were collected with both EDTA concentrations and tested by one operator. A linear regression was conducted with an acceptance criterion of  $r > 0.90$ . The results of the study met the acceptance criterion demonstrating no performance difference between samples collected in either K<sub>2</sub> or K<sub>3</sub>EDTA anticoagulant. Results were as follows:

<b>Hb fraction</b>	<b>n</b>	<b>r<sup>2</sup></b>	<b>y-intercept</b>	<b>slope</b>	<b>Range of values (%)</b>
Hb A	18	1.000	-0.082	1.001	57.2 – 97.9
Hb A2	20	0.999	-0.055	0.984	1.8 – 6.2
Hb F	4	0.999	-0.030	0.998	0.5 – 20.4
Hb S	5	1.000	-0.054	0.999	39.7 – 90.7

3. Clinical studies:

*a. Clinical Sensitivity*

Not applicable

*b. Clinical specificity:*

Not applicable

c. *Other clinical supportive data (when a. and b. are not applicable):*  
Not applicable

4. Clinical cut-off:  
Not applicable

5. Expected values/Reference range:  
Reference values for individual major electrophoretic hemoglobin zones in the CAPILLARYS 2 system were established from an apparently healthy population of 113 adults with normal hemoglobin values using the CAPILLARYS 2 system and the predicate HPLC technique. See results below:  
Hemoglobin A (HbA): comprised between 96.8 and 97.8%  
Hemoglobin F (HbF): < 0.5%  
Hemoglobin A2 (HbA2): comprised between 2.2 and 3.2%  
It is recommended that each laboratory establish its own threshold values.

Normal (reference) values must be considered only when hemoglobin variants are absent.

**N. Instrument Name:**  
Sebia CAPILLARYS 2

**O. System Descriptions:**

1. Modes of Operation:  
Open tube batch mode with the following sequence of automated steps:  
Bar code reading of sample tubes  
Sample hemolysis and dilution from primary tubes  
Capillary washing  
Injection of hemolyzed samples  
Hemoglobin separation and direct detection of the separated hemoglobins on capillaries

2. Software:  
SEBIA PHORESIS operating system software is designed to work with the CAPILLARYS 2 instrument. The CAPILLARYS 2 instrumentation directed by the PHORESIS software is fully automated in the performance of the sample identification by barcode labeling, dilution, testing, and calculation of results. The PHORESIS software, Version 6.50, utilizes *Windows 98 or XP* as the operating system with *Intel* based processors with *Visual Basic* as the programming language.

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes   X   or No \_\_\_\_\_

3. Specimen Identification:

Barcode reader

4. Specimen Sampling and Handling:

Red blood cells are allowed to precipitate at 2° – 8° C or centrifuged at 5000 rpm for 5 minutes. The maximum volume of plasma is removed and the tube is vortexed for 5 seconds. The open tubes are placed in the sample racks.

5. Calibration:

Not applicable

6. Quality Control:

It is necessary to run two analysis sequences with the Normal Hb A2 Control (SEBIA) after having changed buffer lot numbers, after a capillary cleaning, and before starting a new analysis sequence. It is also advised to include into each run of samples an assayed blood control (AFSC Control, Normal Hb A2 Control – SEBIA).

**P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:**

The CAPILLARYS HEMOGLOBIN(E) using the CAPILLARYS 2 instrument is a modification of the previously cleared CAPILLARYS HEMOGLOBIN(E) procedure using the CAPILLARYS instrument (k052291) for separation and detection of Hemoglobin A, F, A2, S, C, E, and D. The BIO-RAD VARIANT™ II β-thalassemia Short Program (k991127) was used as a comparative method for detection of the hemoglobin variants S, C, E and D in this device.

A study was performed using normal, increased HbA2, and pathological to assess if an excess EDTA anticoagulant interferes with analysis. The results obtained indicated that no interference occurs with an excess of EDTA anticoagulant in the collection tube of 4 times higher than the recommended concentration (1.8 mg/dL).

**Q. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**R. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.