

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

A. 510(k) Number:

k112550

B. Purpose for Submission:

New device

C. Measurand:

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

D. Type of Test:

Quantitative, Capillary electrophoresis

E. Applicant:

Sebia Inc.

F. Proprietary and Established Names:

CAPILLARYS HEMOGLOBINE (E) using the CAPILLARYS 2 FLEX-PIERCING 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 FLEX-PIERCING instrument. The CAPILLARYS HEMOGLOBIN(E) kit is designed for laboratory use.

The CAPILLARYS 2 FLEX-PIERCING 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 whole blood samples collected in tubes containing K₂EDTA or K₃EDTA 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 FLEX-PIERCING 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 Flex-Piercing instrument. The CAPILLARYS 2 FLEX-PIERCING instrument has eight capillaries functioning in parallel allowing eight simultaneous analyses for hemoglobin quantification from whole blood samples. The hemoglobin, separated in capillaries, are directly detected at an absorbance wavelength of 415 nm, producing electrophoregrams which are visually evaluated for pattern abnormalities. Direct detection provides relative quantification of individual hemoglobin fractions.

J. Substantial Equivalence Information:

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

3. Comparison with predicate:

Similarities		
Item	Device CAPILLARYS HEMOGLOBIN(E) with CAPILLARYS 2 FLEX-PIERCING instrument	Predicate BIORAD VARIANT™ II β- thalassemia Short Program (k991127)
Intended Use	<p>The CAPILLARYS HEMOGLOBIN(E) kit is designed for the separation of the normal hemoglobins (A, F and A2) in human blood samples, and for the detection of the major hemoglobin variants (S, C, E or D), by capillary electrophoresis in alkaline buffer (pH 9.4) with the SEBIA CAPILLARYS 2 FLEX-PIERCING instrument. The CAPILLARYS HEMOGLOBIN(E) kit is designed for laboratory use.</p> <p>The CAPILLARYS 2 FLEX-PIERCING instrument is an automated analyzer which performs 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 whole blood samples collected in tubes containing K₂EDTA or K₃ EDTA anticoagulant.</p> <p>For in vitro diagnostic use.</p>	<p>The VARIANT™ β-thalassemia Short Program is intended for the separation and area percent determinations of hemoglobins A2 and F and as an aid in the identification of abnormal hemoglobins in whole blood using ion-exchange high performance liquid chromatography (HPLC). The VARIANT™ II β-thalassemia Short Program is intended for use only with the Bio-Rad VARIANT™ II Hemoglobin Test System</p> <p>For in vitro diagnostic use.</p>

Differences		
Item	CAPILLARYS HEMOGLOBIN(E) with CAPILLARYS 2 FLEX PIERCING instrument	BIORAD VARIANT™ II β- thalassemia Short Program (k991127)
Separation system	Free solution capillary electrophoresis (FSCE)	Ion exchange high performance liquid chromatography (HPLC)
Absorbance wavelength	415 nm	415 nm and 690 nm
Number of separation units	8 parallel capillaries	1 parallel capillary
Number of analysis (throughput)	43 samples/hour	9-10 samples/hour
Introduction of sample into automatic system hemolysis	Continuous loading with sample racks	Sequential injection
Controls for migration	SEBIA Normal Hb A2 Control (sold separately)	Hemoglobin A2/F calibrator/diluent set
Use of hemolysis solution	Hemolysis solution is on board	Solution is the VARIANT II sampling solution
Use of other controls	Yes, SEBIA Pathological Hb A2, AFSC and AF Controls)	No
Kit components	All components provided together (boxed).	All components provided separately.
Hb variants library (on-board)	Yes, (displayed by the software and indicated in the package insert)	No

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

- CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods, 2nd Edition
- CLSI EP06-A2: Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach, Approved Guidelines
- CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline. 2nd Edition
- CLSI EP09-A2-1R: Method Comparison and Bias Estimation Using Patient Samples: Approved Guideline. 2nd Edition (Interim Edition)
- CLSI EP17-A: Protocols for the Limit of Detection and Limit of Quantitation; Approved Guideline
- CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents: Approved Guideline
- FDA Use of Symbols on Labels and in Labeling of In-vitro Diagnostic Devices Intended for Professional Use
- FDA Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices - Guidance for Industry and FDA Staff

Test Principle:

The SEBIA CAPILLARYS HEMOGLOBIN(E) test in conjunction with the CAPILLARYS 2 FLEX-PIERCING instrument utilizes the principle of capillary electrophoresis in 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 electro-osmotic flow. 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. Direct detection provides accurate relative quantification of individual hemoglobin fractions: A, F, A2, S, C, D, and E. 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:*

A reproducibility study was performed using seven different blood samples, (4 blood samples and 3 controls identified in the table below). The samples were run using the CAPILLARYS HEMOGLOBIN(E) procedure on the same FLEX-PIERCING instrument using 3 lots of HEMOGLOBIN(E) kits. Each blood sample was run over 27 days, twice a day, and in duplicate for each run. A summary of data and acceptance criteria for the studies performed is found in the tables below. Acceptance criteria were met for HbA, HbA2 and HbS <10% and HbF <20%.

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	Hb AF control
n=120 Sample #	1	2	3	4	5	6	7
Mean Hb%	97.2	95.4	98.1	54.9	97.4	93.8	34.3
Repeatability (CV%)	0.0	0.0	0.0	0.2	0.1	0.1	0.5
Between-run (CV%)	0.0	0.0	0.0	0.21	0.0	0.0	0.6
Between-day (CV%)	0.0	0.0	0.0	0.1	0.0	0.1	0.9
Total (CV%)	0.0	0.1	0.0	0.4	0.1	0.1	1.2
SD	0.05	0.06	0.04	0.20	0.07	0.10	0.42
95% CI	0.04-0.05	0.05-0.07	0.03-0.04	0.17-0.24	0.06-0.08	0.08-0.12	0.34-0.56

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	Hb AF control
n=120 Sample #	1	2	3	4	5	6	7
Mean Hb%	2.8	4.7	1.9	2.8	2.6	6.2	/
Repeatability (CV%)	1.6	1.0	1.5	1.6	2.6	1.3	/
Between-run (CV%)	0.4	0.0	1.2	0.4	0.0	0.6	/
Between-day (CV%)	0.1	0.7	0.0	0.6	0.7	0.6	/
Total (CV%)	1.6	1.2	1.9	1.7	2.6	1.6	/
SD	0.05	0.06	0.04	0.05	0.07	0.10	/
95% CI	0.04-0.05	0.05-0.07	0.03-0.04	0.04-0.06	0.06-0.08	0.08-0.12	/

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	Hb AF control
n=120 Sample #	1	2	3	4	5	6	7
Mean Hb%	/	/	/	8.3	/	/	65.2
Repeatability (CV%)	/	/	/	0.7	/	/	0.3
Between-run (CV%)	/	/	/	0.8	/	/	0.4
Between-day (CV%)	/	/	/	0.3	/	/	0.5
Total (CV%)	/	/	/	1.1	/	/	.07
SD	/	/	/	0.09	/	/	0.42
95% CI	/	/	/	0.08-0.11	/	/	0.34-0.56

Acceptance criteria: %CV <10%

HbS study reproducibility summary

HbS %	Normal blood	Blood sample with elevated Hb A2	Blood with low HbA2 level	Pathological blood with HbF & HbS	Normal HbA2 control	Pathological Hb A2 control	Hb AF control
n=120 Sample #	1	2	3	4	5	6	7
Mean Hb%	/	/	/	33.9	/	/	/
Repeatability (CV%)	/	/	/	0.4	/	/	/
Between-run (CV%)	/	/	/	0.4	/	/	/
Between-day (CV%)	/	/	/	0.1	/	/	/
Total (CV%)	/	/	/	0.6	/	/	/
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 FLEX PIERCING 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%	97.2	95.3	98.1	55.0	97.3	93.5	33.3
Between-instrument (CV%)	0.1	0.0	0.0	0.3	0.1	0.1	0.4
Total (CV%)	0.1	0.1	0.0	0.4	0.1	0.3	1.9
SD	0.06	0.08	0.04	0.24	0.13	0.24	0.64
95% CI	0.06-0.07	0.06-0.09	0.04-0.05	0.20-0.29	0.10-0.16	0.19-0.31	0.51-0.84

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	Hb AF control
n=48 Sample #	1	2	3	4	5	6	7
Mean Hb A%	2.8	4.7	1.9	2.8	2.7	6.5	/
Between-instrument (CV%)	2.0	0.9	2.1	1.5	2.2	1.2	/
Total (CV%)	2.3	1.6	2.2	1.8	4.6	0.7	/
SD	0.06	0.08	0.04	0.05	0.13	0.24	
95% CI	0.06-0.07	0.06-0.09	0.04-0.05	0.04-.06	0.10-0.16	0.19-0.31	/

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 A%	/	/	/	8.3	/	/	65.2
Between-instrument (CV%)	/	/	/	0.6	/	/	0.2
Total (CV%)	/	/	/	1.1	/	/	
SD	/	/	/	0.09	/	/	0.60
95% CI	/	/	/	0.17-0.24	/	/	0.48-0.79

Acceptance criteria: %CV <10%

Between-instrument reproducibility study summary for HbS

HbS %	Normal blood	Blood sample with elevated HbA2	Blood with low HbA2 level	Pathological blood with Hb F & Hb S	Normal HbA2 control	Pathological HbA2 control	Hb AF control
n=48 Sample #	1	2	3	4	5	6	7
Mean Hb A%	/	/	/	33.9	/	/	/
Between-instrument (CV%)	/	/	/	0.3	/	/	/
Total (CV%)	/	/	/	0.6	/	/	/
SD	/	/	/	0.08	/	/	/
95% CI	/	/	/	.06-0.12	/	/	/

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 FLEX PIERCING instrument. Nine different samples (three samples per hemoglobin variant) containing HbC, HbD or HbE were analyzed on three different CAPILLARYS 2 FLEX PIERCING 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

	Sample C1	Sample C2	Sample C3
Hb C % n=48	Pathological Sample with HbC	Pathological Sample with HbC	Pathological Sample with HbC
Mean Hb%	44.9	31.8	69.4
Repeatability (CV%)	1.3	1.3	0.5
Between-run (CV%)	0.7	0.0	1.0
Between-day (CV%)	1.3	1.1	0.8
Total Between-instrument	2.0	1.7	1.4
SD/CV%	0.64	0.71	1.09
95% CI	0.73-1.33	0.73-1.33	0.73-1.33

Acceptance criteria: %CV <10%

Hb D reproducibility study summary

	Sample D1	Sample D2	Sample D3
Hb D % n=48	Pathological Sample with Hb D	Pathological Sample with Hb D	Pathological Sample with Hb D
Mean Hb%	40.8	39.0	36.5
Repeatability (CV%)	0.3	0.3	0.4
Between-run (CV%)	0.1	0.2	0.3
Between-day (CV%)	0.9	0.8	1.1
Total Between-instrument	0.9	0.9	1.2
SD/CV%	0.20	0.20	0.26
95% CI	0.32 - 0.70	0.32 - 0.70	0.32 - 0.70

Acceptance criteria: %CV <10%

Hb E reproducibility study summary

	Sample E1	Sample E2	Sample E3
Hb E % n=48	Pathological Sample with Hb E	Pathological Sample with Hb E	Pathological Sample with Hb E
Mean Hb%	25.1	23.9	22.9
Repeatability (CV%)	0.7	0.5	0.7
Between-run (CV%)	0.4	0.3	0.5
Between-day (CV%)	0.5	0.5	1.0
Total Between-instrument	0.9	0.8	1.3
SD/CV%	0.20	0.40	0.29
95% CI	0.23 - 0.43	0.23 - 0.43	0.23 - 0.43

Acceptance criteria: %CV <10%

The reproducibility results of the CAPILLARYS HEMOGLOBIN(E) assay kit with the CAPILLARYS 2 FLEX PIERCING instrument met acceptance criteria for all hemoglobins tested (Hb A, HbA2, Hb F, Hb S, Hb C, Hb D and Hb E).

b. Linearity/assay reportable range:

Mixtures of two different blood samples (normal blood with hemoglobin fraction A, A2, S, F and umbilical cord samples) was serially diluted 12 times with saline and analyzed using the CAPILLARYS HEMOGLOBIN(E) assay kit using the CAPILLARYS 2 FLEX PIERCING instrument. A separate linearity study was performed using different pathological blood samples with hemoglobin fraction C, D and E and serially diluted 11 times with normal whole and analyzed using the CAPILLARYS HEMOGLOBIN(E) assay kit. The linearity study was performed 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: 0-97.5%, HbA2: 0-9.9%, HbF: 0-75.5%, HbS: 0-90.7%, HbC: 0-33.2%, HbD: 0-40.9% and HbE: 0-96.1%.

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 conducted on three different whole blood samples: a normal sample, an increased HbA2 level sample, and a pathological sample with abnormal HbS. Each sample was prepared as follows 1) whole blood, 2) its own hemolysate re-suspended with its own plasma, 3) its own hemolysate re-suspended and spiked with a pre-determined amount of interferents. Each sample was analyzed 3 times to quantitatively detect hemoglobin variants A, A2, F and S for reproducibility. Study results indicate the maximum concentration for each interferent as follows:

Interferents	Maximum Concentration
Bilirubin	17.9 mg/dL
Lipemia	22.34 g/dL (triglycerides)

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 using the CAPILLARYS FLEX 2 PIERCING instrument and compared to the predicate, BIO-RAD assay kit with the β -Thalassemia Short Program system, at three sites on blood samples with normal and abnormal levels for HbA, HbA2, HbF, HbS and hemoglobin variants C, D and E. The blood samples and their 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 ≤ 1 year to adult (≥ 21). The CAPILLARYS 2 FLEX-PIERCING instrument was not evaluated in the newborn/neonate population. The acceptance criterion was defined as the correlation coefficient (r^2) > 0.90 . A regression analysis was calculated producing a slope, intercept and correlation coefficient (r^2) 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: The internal study 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 ²	y-intercept	Slope	95% CI	Range of values (%)
Hb A	56	0.996	-11.42	1.33	-13.52 to -9.32	21.0 – 98.2
Hb A2	44	0.978	-0.08	1.13	-0.14 to -0.02	0.1 – 6.3
Hb F	56	1.000	-0.38	0.93	-0.48 to 0.27	0.00 – 79.0
Hb S	8	0.998	-0.06	1.07	-0.79 to 0.91	6.8 – 41.2

Acceptance criteria: r² = >90%

Note: Additional data and supplemental statistical analyses were requested from the sponsor to assess the high y-intercept value for HbA at site 1. A higher overall test result for %HbA in the new device when compared to the predicate was noted. Statistical review of the combined data for HbA at all sites indicated that this variant demonstrated a good fit to the linear model, with R² 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.

Site 1 included samples with 20 abnormal variants. Two HbC, four HbD and four HbE variants were detected and were in agreement with the comparative HPLC system and clinical diagnosis.

Site 2: The first external site 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 ²	y-intercept	slope	95% CI	Range of values (%)
Hb A	121	0.999	-1.71	1.14	1.13 to 1.14	0.0 – 98.8
Hb A2	93	0.986	-0.31	1.16	1.12 to 1.20	0.4 – 6.5
Hb F	103	0.997	-0.65	0.97	0.95 to 0.98	0.0 – 90.3
Hb S	26	0.998	-0.10	1.05	1.02 to 1.08	14.2 – 54.8
Hb C	5	0.999	-1.08	1.02	0.95 to 1.10	9.7 – 44.7

Acceptance criteria: r² = >90%

Site 2 included samples with 33 abnormal variants. Five HbC and two HbE were detected and were in agreement with the comparative HPLC system and clinical diagnosis.

Site 3: The second external site 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). Results were as follows:

Hb fraction	n	r²	y-intercept	slope	95% CI	Range of values (%)
Hb A	180	0.991	-1.75	1.13	1.11 to 1.15	0.9 – 91.0
Hb A2	113	0.926	-0.18	1.04	0.96 to 1.11	0.4 – 6.0
Hb F	181	0.991	-0.11	1.06	1.04 to 1.08	0.0-98.2
Hb S	67	0.996	0.66	1.0	0.98 to 1.02	5.0-93.9
Hb C	13	0.996	0.81	0.94	0.88 to 0.99	12.5-45.7

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

Site 3 included samples with 83 abnormal hemoglobin variants. Thirteen HbC and three HbD variants were detected and were in agreement with the comparative HPLC system and clinical diagnosis.

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 and clinical diagnosis. There were no observed false positives (i.e., detection of an abnormal band or abnormal level of a normal band where no such abnormal existed).

b. Matrix comparison:

A study was performed with K₂EDTA and K₃EDTA using normal, increased HbA₂, and pathological samples (with HbF and HbS) to determine whether an excess of EDTA anticoagulant interferes with analysis. The results obtained indicated no interference with an excess of EDTA anticoagulant in the collection tube of 4 times higher than the recommended concentration (1.8 mg/dL). Testing of the samples was performed on the CAPILLARYS 2 FLEX-PIERCING instrument using 3 lots of CAPILLARYS HEMOGLOBIN(E) assay kit. A total of 20 (15 normal and 5 abnormal) samples were tested. The same samples were collected with both EDTA K₂ and K₃ anticoagulants and tested by one operator for quantitative detection of hemoglobin variants. Linear regression was performed on the test results. The acceptance criterion is $r^2 >0.90$. The results of the study met the acceptance criterion demonstrating no performance difference between samples collected in either EDTA concentration. Results of the study were as follows:

Hb fractions	n	r ²	y-intercept	slope	Range of Hb% variants
Hb A	18	1.009	0.638	0.993	57.5 - 98.0
Hb A2	20	0.997	-0.009	1.007	1.8 – 6.2
Hb F	4	1.000	-0.216	1.043	0.7 – 20.1
Hb S	5	1.000	-0.637	1.009	39.5 – 91.3

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 with the HEMOGLOBIN(E) assay kit and the CAPILLARYS 2 FLEX-PIERCING instrument was established from a healthy population of 113 adults with normal hemoglobin values using the CAPILLARY 2 FLEX-PIERCING instrument and the HPLC technique. Results are found in the table below:

Hemoglobin Variants	Reference range
Hemoglobin A (Hbg A)	96.8 and 97.8%
Hemoglobin F (Hbg F)	< 0.5 %
Hemoglobin A2 (Hbg A2)	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 FLEX-PIERCING instrument

O. System Descriptions:

1. Modes of Operation:

Closed tube batch mode with the following automated steps:

Bar code reading of sample tubes (for up to 8 tubes) and sample racks

Mixing of blood before analysis

Sample hemolysis and dilution from primary tubes into dilution segments

Capillary washing

Injection of hemolyzed samples

Hemoglobin separation and direct detection of the separated hemoglobins on capillaries

2. Software:

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

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

Yes 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 closed or 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 FLEX-PIERCING 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 with this device.

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 substantial equivalence decision.