510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K192931

B Applicant

Helena Laboratories, Corp.

C Proprietary and Established Names

V8 Nexus Hemoglobin UltraScreen, V8 AFSA2 Hemo Control

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
GKA	Class II	21 CFR 864.7415 - Abnormal Hemoglobin Assay	HE - Hematology
JBD	Class II	21 CFR 864.7440 – Electrophoretic Hemoglobin Analysis System	HE - Hematology

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Hemoglobin A, F, A2, S, C

C Type of Test:

Capillary Zone Electrophoresis

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use

B Indication(s) for Use:
The V8 Nexus Hemoglobin UltraScreen method is designed for the separation of normal hemoglobins (A, A2, and F) in human blood samples, and for the detection of major hemoglobins variants (S and C) by using a capillary zone electrophoresis (CZE) buffer with the V8 instrument. The V8 Nexus Hemoglobin UltraScreen test is indicated for use in patients 2 years of age and older. This test is designed for in-vitro diagnostic use only in conjunction with other laboratory and clinical findings.

The V8 instrument is an automated analyzer which performs a complete hemoglobin profile for quantitative analysis of the normal hemoglobin fractions A, A2 and F and for the detection of major hemoglobin variants S and C. The assay is performed on the hemolysate of venous whole blood collected in tubes containing K2EDTA as the anticoagulant.

The V8 AFSA2 Hemo Control (Cat. No. 1812) is to be used as a quantitative and/or qualitative control for the Hemoglobin UltraScreen on the V8 Capillary Electrophoresis (CE) System.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:

For use with Helena V8 Capillary Electrophoresis System (K111369)

IV Device/System Characteristics:

A Device Description:

The V8 Nexus Hemoglobin UltraScreen kit is designed for use on the V8 Nexus Capillary Electrophoresis (CE) system. The kit contains sufficient reagent to perform 200 tests, and it includes the following components: V8 Nexus Hemoglobin UltraScreen Buffer, V8 Nexus Hemoglobin UltraScreen Diluent, V8 Filter, and V8 Sample Cups.

The V8 Nexus Hemoglobin UltraScreen kit is designed for the separation of normal hemoglobin (A, A2, and F) in human blood samples, and for the detection of major hemoglobin variants (S and C) using the automated analyzer V8 Capillary Electrophoresis (CE) System.

The V8 AFSA2 Hemo Control is to be used as a quantitative and/or qualitative control for the V8 Nexus Hemoglobin UltraScreen method on the V8 Capillary Electrophoresis (CE) System. It can be used to verify all phases of the Hemoglobin UltraScreen procedure.

B Principle of Operation:

The V8 Capillary Electrophoresis (CE) System is a counter-top electrophoresis system capable of separating normal hemoglobin fractions A, A2 and F as well as hemoglobin variants S and C.

Capillary zone electrophoresis is achieved using a microbore, fused silica capillary filled with an electrolyte medium under high voltage. Separation of the charged analytes is due to differential migration in an electrical field, whereby the charged particles migrate toward an electrode with an opposite charge. Using V8 Nexus Hemoglobin UltraScreen kit with capillary zone electrophoresis method available on the V8 Capillary Electrophoresis (CE) System will separate the more common variants from the normal A peak. The run positions of variant hemoglobins can be used to give an initial identification of the variants.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Sebia CAPILLARYS HEMOGLOBIN(E) with CAPILLARYS 2 Instrument

B Predicate 510(k) Number(s):
K112491

C Comparison with Predicate(s):

Device & Predicate Device(s):	K192931	K112491
Device Trade Name	**Helena Laboratories V8 Nexus Hemoglobin UltraScreen Test**	**Sebia CAPILLARYS Hemoglobin(E) Test**
General Device Characteristic Similarities		
Type of Test	Quantitative, Capillary Zone Electrophoresis	Quantitative, Capillary Electrophoresis
Intended User	Clinical Laboratory Professional	Same
Specimen Type	Hemolysate of venous whole blood	Same
Sample Preparation	Sample hemolysis performed automatically by the instrument	Same
Absorbance Wavelength	415 nm	Same
General Device Characteristic Differences		
Instrument	V8 Instrument	CAPILLARYS 2 Instrument
Measurand	Hemoglobin A, F, A2, S, C	Hemoglobin A, F, A2, S, C, E, D
Intended Use	*In vitro* diagnostic hemoglobin test for the quantitative detection of individual hemoglobin fractions A, A_2 and F and qualitative detection of major hemoglobin variants S and C from EDTA anti-coagulated human whole blood	*In vitro* diagnostic hemoglobin test for the quantitative detection of individual hemoglobin fractions A, A_2 and F and qualitative detection of major hemoglobin variants S, C, E and D from EDTA anti-coagulated

	specimens.	human whole blood specimens.
Anticoagulant	K_2EDTA	K_2EDTA and K_3EDTA
Technological Detection Principles	Free solution capillary electrophoresis – protein separation occurs in an electrolyte medium, according to their electrophoretic mobilities. Electropherograms show separated fractions based on mass to charge ratio.	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. Electropherograms show separated fractions according to their charge.
Control for Migration / Other	V8 AFSA2 Hemo Control	Normal Hb A2 control; Hb AFSC

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition

CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition.

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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
   a) 20-day precision study using AFSA2 Hemo control: A 20-day in-house precision study was performed on the V8 Capillary Electrophoresis (CE) System (V8 instrument) with V8 Nexus Hemoglobin UltraScreen kit (reagent kit) using AFSA2 Hemo control. The study used one lot of reagent kit, one lot of AFSA2 Hemo control, one instrument, and one operator. The tests were run in a design of 20 days, two runs per day, and eight replicates per run, for a total of 320 determinations per hemoglobin fraction. Between-run, between-day, within-capillary, between-capillary, and total precision were determined. All precision estimates for all Hb fractions were within the acceptance criteria.

	N	Mean %	Within-Capillary		Between-Capillary		Between-Run		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
A	320	43.2	0.13	0.3	1.64	3.8	0.39	0.9	0.39	0.9	1.77	4.1
F	320	33.1	0.10	0.3	1.09	3.3	0.26	0.8	0.26	0.8	1.13	3.4
S	320	21.7	0.15	0.7	0.09	0.4	0.22	1.0	0.22	1.0	0.37	1.7
A2	320	1.9	0.07	3.5	0.03	1.5	0.07	3.6	0.07	3.7	0.11	5.6

b) 20-day precision study using AFSC hemoglobin control: A 20-day in-house precision study was performed on the V8 instrument with V8 Nexus Hemoglobin UltraScreen kit (reagent kit) using AFSC hemoglobin control. The study used one lot of reagent kit, one lot of AFSC control, one V8 instrument, and one operator. The tests were run in a design of 20 days, two runs per day, and eight replicates per run, for a total of 320 determinations per hemoglobin fraction. Between-run, between-day, within-capillary, between-capillary, and total precision were determined. All precision estimates for all Hb fractions are within the acceptance criteria.

Hb Fraction	N	Mean %	Within-Capillary		Between-Capillary		Between-Run		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
A	320	29.3	0.09	0.3	0.38	1.3	0.18	0.6	0.18	0.6	0.47	1.6
F	320	24.1	0.07	0.3	0.51	2.1	0.17	0.7	0.19	0.8	0.60	2.5
S	320	25.0	0.10	0.4	0.13	0.5	0.15	0.6	0.15	0.7	0.22	1.3
C	320	21.6	0.11	0.5	0.04	0.2	0.15	0.7	0.15	0.7	0.22	1.0

c) Reagent lot-to-lot precision study using AFSA2 Hemo control: An internal precision study was performed using three lots of V8 Hemoglobin UltraScreen reagent kits, one lot of AFSA2 control, one V8 instrument, and one operator. The tests were run over five days, two runs per day, eight capillary determinations (8 replicates) per run, for a total of 80 determinations per reagent lot, or 240 determinations per hemoglobin fraction across three reagent lots. Within-run, between run, between-day, between-lot and total precision were calculated. All hemoglobin fractions met acceptance criteria demonstrating acceptable reagent lot to lot precision.

Hb Fraction	N	Mean %	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
A	240	42.9	0.26	0.6	1.63	3.8	0.26	0.9	0.43	1.2	1.72	4.0
F	240	33.3	0.17	0.5	1.03	3.1	0.20	0.8	0.27	1.0	1.10	3.4
S	240	21.8	0.26	1.2	0.57	2.6	0.37	1.8	0.57	2.9	0.92	4.4
A2	240	2.0	0.07	3.5	0.07	3.4	0.09	6.7	0.09	6.7	0.14	8.9

d) Control lot-to-lot precision study using AFSA2 Hemo control: An internal precision study was performed using three lots of AFSA2 Hemo control, one lot of V8 Nexus Hemoglobin UltraScreen reagent kit, one V8 instrument, and one operator. The tests were run over 20 days, two runs per day, eight capillary determinations (8 replicates) per run, for a total of 320 determinations per control lot, or 960 determinations per hemoglobin fraction across three control lots. Within-run, between run, between-day, between-lot and total precision were calculated. All hemoglobin fractions met acceptance criteria demonstrating acceptable AFSA2 control lot-to-lot precision.

Hb Fraction	N	Mean %	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
A	960	43.2	0.18	0.4	1.31	3.0	0.50	1.2	0.49	1.1	1.44	3.3
F	960	33.1	0.15	0.7	0.92	4.2	0.35	1.6	0.35	1.6	1.02	4.7
S	960	21.7	0.22	0.7	0.66	2.0	0.32	1.0	0.31	0.9	0.78	2.4
A2	960	1.9	0.07	3.5	0.09	4.8	0.07	3.8	0.07	3.8	0.14	7.2

e) Instrument precision study using patient samples: A precision study was performed using fresh and refrigerated venous K_2EDTA patient samples with known hemoglobin variants. Normal Hb AA2, Hb AF (elevated F), Hb ASA2 (elevated S and A2) and Hb AA2C (elevated C and A2) were analyzed over the course of the 5-day study. Study was conducted at an internal site, with one lot of V8 Nexus Hemoglobin UltraScreen reagent kit, three V8 instruments, and one operator. The tests were run over five days, two runs per day, and eight capillary determinations (8 replicates) per run, for a total of 80 determinations per sample per instrument, or 240 determinations across three instruments. Within-run, between-run, between-day, between-instrument and total precision were calculated. All hemoglobin fractions met acceptance criteria, demonstrating acceptable precision across instruments.

Sample Hb A	N	Mean %	Within-Run		Between-Run		Between-Day		Between-Instrument		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	240	96.9	0.0	0.0	0.0	0.0	0.10	0.1	0.10	0.1	0.10	0.1
2	240	34.8	0.35	1.0	0.66	1.9	0.42	1.2	0.42	1.2	0.87	2.5
3	240	61.0	0.37	0.6	0.12	0.2	0.43	0.7	0.67	1.1	0.79	1.3
4	240	58.0	0.35	0.6	0.29	0.5	0.58	1.0	0.70	1.2	0.93	1.6

Sample Hb F	N	Mean %	Within-Run		Between-Run		Between-Day		Between-Instrument		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
2	240	65.2	0.33	0.5	0.65	1.0	0.46	0.7	0.39	0.6	0.85	1.3

Sample Hb A2	N	Mean %	Within-Run		Between-Run		Between-Day		Between-Instrument		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	240	3.1	0.05	1.5	0.00	0.1	0.05	1.6	0.05	1.6	0.07	2.2
3	240	3.6	0.05	1.3	0.01	0.4	0.05	1.3	0.05	1.3	0.07	1.9
4	240	5.5	0.07	1.2	0.06	1.1	0.07	1.2	0.07	1.2	0.12	2.1

Sample Hb S	N	Mean %	Within-Run		Between-Run		Between-Day		Between-Instrument		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
3	240	34.5	0.35	1.0	0.17	0.5	0.38	1.1	0.59	1.7	0.76	2.2

Sample Hb C	N	Mean %	Within-Run		Between-Run		Between-Day		Between-Instrument		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
4	240	36.5	0.37	1.0	0.29	0.8	0.58	1.6	0.73	2.0	0.95	2.6

f. 5-Day multi-site reproducibility study using patient samples:
Site-to-site precision of the V8 Nexus Hemoglobin UltraScreen assay was assessed at three different sites, including one internal laboratory and two external clinical laboratories. One V8 instrument, one lot of V8 Nexus Hemoglobin UltraScreen reagent kit (same at all 3 sites) and a single operator per site were utilized. The tests were run for five days, with two runs per day and each run consisted of four replicates (3 sites x 5 days x 2 runs/day x 4 replicates per run). Each sample has 120 total determinations per hemoglobin fraction. Within-run, between-run, between-day, between-site and total precision were analyzed.

Nine samples of varying level of hemoglobin A, A2, F, S and C were prepared by controlled proportion mixing to generate hemoglobin percentages for each fraction in the normal and pathologic range, where applicable.

Overall, reproducibility which includes within-run, between-run, between-day, and between-site components for all hemoglobin fractions quantitated over the course of the 5-day study met predetermined acceptance criteria.

Hb A Sample	N	Mean %	Within-Run		Between-Run		Between-Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	120	97.2	0.00	0.0	0.00	0.0	0.10	0.10	0.10	0.1	0.10	0.1
2	120	74.7	0.30	0.4	0.52	0.7	0.37	0.5	0.45	0.6	0.82	1.1
3	120	87.4	0.26	0.3	0.26	0.3	0.26	0.3	0.44	0.5	0.61	0.7
4	120	59.3	0.18	0.3	0.24	0.4	0.18	0.3	0.18	0.3	0.36	0.6
5	120	76.6	0.23	0.3	0.00	0.0	0.23	0.3	0.38	0.5	0.54	0.7
6	120	84.9	0.25	0.3	0.00	0.0	0.34	0.4	0.51	0.6	0.76	0.9
7	120	57.1	0.34	0.6	0.06	0.1	0.34	0.6	0.34	0.6	0.51	0.9
8	120	78.9	0.24	0.3	0.32	0.4	0.32	0.4	0.32	0.4	0.51	0.6
9	120	87.9	0.18	0.2	0.26	0.3	0.18	0.2	0.26	0.2	0.44	0.5
Hb A2 Sample	**N**	**Mean %**	**Within-Run**		**Between-Run**		**Between-Day**		**Between-Site**		**Total**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	120	2.8	0.03	1.1	0.02	0.7	0.03	1.1	0.03	1.1	0.05	1.8
2	120	2.0	0.03	1.5	0.01	0.5	0.03	1.5	0.03	1.5	0.05	2.5
3	120	2.5	0.05	2.0	0.06	2.4	0.05	2.0	0.05	2.0	0.09	3.6
4	120	3.5	0.03	0.9	0.0	0.0	0.03	0.9	0.03	0.9	0.04	1.1
5	120	3.0	0.04	1.3	0.01	0.4	0.04	1.3	0.04	1.3	0.06	2.0
6	120	3.2	0.05	1.6	0.04	1.3	0.05	1.6	0.05	1.6	0.08	2.5
7	120	5.6	0.06	1.1	0.07	1.3	0.06	1.1	0.06	1.1	0.11	2.0
8	120	4.0	0.04	1.0	0.01	0.3	0.04	1.0	0.04	1.0	0.06	1.5
9	120	3.5	0.05	1.4	0.02	0.6	0.05	1.4	0.05	1.4	0.07	2.0
Hb F Sample	**N**	**Mean %**	**Within-Run**		**Between-Run**		**Between-Day**		**Between-Site**		**Total**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV

2	120	23.3	0.33	1.4	0.58	2.5	0.35	1.5	0.42	1.8	0.82	3.5
3	120	10.1	0.23	2.3	0.25	2.5	0.27	2.7	0.39	3.9	0.62	6.1
Hb S Sample	**N**	**Mean %**	**Within-Run**		**Between-Run**		**Between- Day**		**Between-Site**		**Total**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
4	120	37.2	0.19	0.5	0.26	0.7	0.19	0.5	0.19	0.5	0.37	1.0
5	120	20.2	0.20	1.0	0.04	0.2	0.24	1.2	0.36	1.8	0.51	2.5
6	120	12.1	0.21	1.7	0.05	0.4	0.31	2.6	0.56	4.6	0.74	6.1
Hb C Sample	**N**	**Mean %**	**Within-Run**		**Between-Run**		**Between-Day**		**Between-Site**		**Total**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
7	120	37.3	0.34	0.9	0.07	0.2	0.34	0.9	0.37	1.0	0.52	1.4
8	120	17.1	0.24	1.4	0.29	1.7	0.31	1.8	0.32	1.9	0.53	3.1
9	120	8.6	0.15	1.7	0.33	3.8	0.21	2.4	0.24	2.8	0.46	5.3

2. Linearity:

The linearity studies were performed using one V8 Nexus Hemoglobin UltraScreen reagent kit and one V8 instrument, and venous K2-EDTA samples containing different levels of each hemoglobin fraction (9 levels for each fraction). The samples were allowed to reach room temperature prior to testing. Linearity was determined by comparing the assigned hemoglobin fraction % to the measured fraction % for each sample. The absolute concentration of hemoglobin fractions A, A2, F, S and C varied by using neat undiluted sample or sample mixing at specific ratios.

Hemoglobin A is linear from 3.7 – 97.2%.
Hemoglobin A2 is linear from 1.7 – 7.6%.
Hemoglobin F is linear from 1.1 – 68.7%.
Hemoglobin S is linear from 5.8 – 78.8%.
Hemoglobin C is linear from 1.4 – 42.6%.

3. Analytical Specificity/Interference:

Lipids, conjugated bilirubin, and unconjugated bilirubin interference

Interference studies were conducted using venous K_2EDTA whole blood samples that included five Hb A levels, four Hb A2 levels (two normal and two elevated), two Hb F levels, one Hb S level, and one Hb C level. Lipids, unconjugated bilirubin, and conjugated bilirubin were analyzed for interfering with hemoglobin quantitation. Lipids were tested at 0–25 g/L. Unconjugated and conjugated bilirubin were tested at 0–25 mg/dL. The concentrations for bilirubin were 0, 5, 10, 15, 20, and 25 mg/dL and for lipids were 0, 5, 10, 15, 20, and 25 g/L. Each hemoglobin fraction level was analyzed four times (four replicates) per interferent level. Unconjugated bilirubin, conjugated bilirubin, and lipids were found not to affect test performance at concentrations up to 25 mg/dL and 25 g/L, respectively.

Interferent	Maximum Concentration
Lipids	25 g/L
Unconjugated Bilirubin	25 mg/dL
Conjugated Bilirubin	25 mg/dL

K2-EDTA interference

An interference study was conducted to assess the interference of K_2EDTA with respect to hemoglobin quantitation. The study used fresh venous K_2EDTA whole blood samples that included three Hb A levels, three Hb A2 levels (1 normal and 2 elevated), and one level of Hb S and Hb C, respectively. K_2EDTA was tested at 3.6 mg/mL (normal) and 7.2 mg/mL (elevated). No interference by K_2EDTA was observed at concentration up to 7.2 mg/mL

4. Assay Reportable Range:

For each hemoglobin fraction, the reportable range of the V8 Nexus Hemoglobin UltraScreen procedure performed using the V8 Capillary Electrophoresis (CE) instrument is summarized below:

Fraction	Reportable Range
Hb A	3.7 – 97.2%
Hb A2	1.7 – 7.6%
Hb F	1.1 – 68.7%
Hb S	5.8 – 78.8%
Hb C	1.4 – 42.6%

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) Reagent Stability using normal and abnormal AA2 controls: Reagent stability study was conducted using three lots of Hemoglobin UltraScreen reagents, normal AA2 assayed control, and abnormal AA2 assayed control (controls manufactured by Canterbury Scientific), with five replicates per control per time point. The study, for reagent stability stored at room temperature, was conducted over 24 months with intervals at 0, 1, 3, 6, 9, 12, 18 and 24 months. Overall, all data met acceptable criteria, demonstrating that Hemoglobin UltraScreen reagents are stable for up to 24 months. A reagent stability of 18 months at room temperature (15˚C – 30˚C) was claimed.

b) Open Reagent Stability using AA2 controls: Open reagent stability was assessed using three lots of the Hemoglobin UltraScreen reagents. One lot each of normal AA2 assayed control and abnormal AA2 assayed control was analyzed. The controls were commercially available (manufactured by Canterbury Scientific). Each lot of Hemoglobin UltraScreen reagent was assayed over a 6-month period at defined time points (0 (T0), 1, 2, 3, 4, 5, 6 months). At time zero, the reagents were opened, and assays were performed. The reagents were sealed and stored at room temperature (15˚C – 30˚C, recommended storage conditions). The same protocol was employed for each time point. Five replicates were run per time point for each hemoglobin control used. Overall, all data met acceptable criteria, demonstrating that opened Hemoglobin UltraScreen reagents are stable for up to 6 months. An open reagent stability of 5 months at room temperature (15˚C – 30˚C) was claimed.

c) Reagent Stability using AFSA2 control and Hb C donor sample: Reagent stability was assessed using three lots of Hemoglobin UltraScreen reagents and a single lot of AFSA2

hemoglobin control. The AFSA2 control contains hemoglobins A, F, S and A2 representing each of the four major intervals of migration. Hemoglobin A represents migration interval Hb A. Hemoglobin F represents migration interval Hb F. Hemoglobin S represents migration interval Hb S/D and Hb A2 represents migration interval Hb A2/C/E. Each lot of Hemoglobin UltraScreen reagent was assayed over a 22-month period at defined time points (0, 3, 5, 7, 9, 11, 12, 13, 18 and 22 months). At months 18 and 22, a Hb C patient sample was analyzed on the three lots of Hemoglobin UltraScreen reagent compared to a recently manufactured Hemoglobin UltraScreen lot for comparison (5 months post manufacture). Five replicates were run per time point for each lot of Hemoglobin UltraScreen kit used. Overall, all data met acceptable criteria, demonstrating that Hemoglobin UltraScreen reagents are stable for 22 months. The Hb C sample quantitation was identical on three lots of Hemoglobin UltraScreen reagents at 18 and 22 months compared to a newly manufactured lot. A reagent stability of 18 months at room temperature (15˚C – 30˚C) was claimed.

d) AFSA2 Control Shelf Life Stability: AFSA2 hemoglobin control stability was assessed using three lots of AFSA2 hemoglobin control and one lot of Hemoglobin UltraScreen reagents. Each lot of AFSA2 hemoglobin control was evaluated over 22 months at defined time points (0 (T0), 3, 5, 7, 9, 11, 12, 13, 18 and 22 months). A new vial of each control lot was used at each time point. Five replicates were run for each lot at each defined time point. All data were within acceptable criteria. AFSA2 hemoglobin controls were demonstrated to be stable for 22 months. A stability of 18 months at refrigerated temperature (2˚C – 8˚C) was claimed.

e) AFSA2 Control Open Vial Stability: An open-vial stability assessment was performed for AFSA2 hemoglobin control. Three lots of AFSA2 hemoglobin control and one lot of Hemoglobin UltraScreen reagents were utilized. Each lot of AFSA2 control was assayed over 8 days with defined time points (0 (T0), 3, 6, 7, 8 days). All vials of AFSA2 hemoglobin controls were opened at day 0 and three vials assayed per lot. Unused vials were returned to 2–8 ˚C storage. At each time point, three previously opened vials per lot were equilibrated to room temperature and assayed. Three replicates from each of three vials were run per time point for each hemoglobin control lot used. All data were within acceptable criteria. AFSA2 hemoglobin controls were demonstrated to be stable for 8 days after opening followed by refrigerated storage. An open vial stability of 7 days at refrigerated temperature (2˚C – 8˚C) was claimed.

6. Detection Limit:
   a) Limit of Blank (LoB): LoB was determined using the V8 Nexus Hemoglobin UltraScreen assay on the V8 Capillary Electrophoresis (CE) System over the course of two days. Two lots of V8 Nexus Hemoglobin UltraScreen and four blank samples containing no hemoglobin were used. The LoB was calculated as LoB = mean of blank + 1.645(SD of blank). In the absence of hemoglobin containing sample, the UltraScreen assay yields no quantitative hemoglobin signal, and thus LoB = 0.

   b) Limit of Detection (LoD) and Limit of quantitation (LoQ): LoD and LoQ were combined and were defined by the lower limit of linearity. Since the acceptance criteria for the lower limits of linearity for each hemoglobin fraction satisfied the requirements for

LoD/LoQ, the lower limit of linearity for each hemoglobin fraction was assigned as the LoD/LoQ for that fraction.

Fraction	LoD/LoQ
Hb A	3.7%
Hb A2	1.7%
Hb F	1.1%
Hb S	5.8%
Hb C	1.4%

7. Assay Cut-Off:
Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was conducted at three external sites (1 OUS and 2 US sites) comparing the results from the V8 Nexus Hemoglobin UltraScreen test to that of Sebia's Capillarys Hemoglobin(E) Test (K112491). Each site used a different lot of V8 Nexus Hemoglobin UltraScreen reagent kit (3 in total). Fresh venous K_2EDTA anticoagulated whole blood samples were used. From a total of 439 patient samples, 320 hemoglobin A, 412 hemoglobin A2, and 175 hemoglobin F quantitation's were performed. 143 presumptive hemoglobin S and 33 presumptive hemoglobin C were also performed. A single data point was collected per device platform. UltraScreen data points were accepted only if the data points were within the UltraScreen linearity range established for each hemoglobin fraction.

For each hemoglobin fraction, Deming regression, Passing-Bablok regression and Bland-Altman analysis were performed for Helena V8 Nexus Hemoglobin UltraScreen versus Sebia Capillarys Hemoglobin(E) Test results. Results for the individual sites as well as all sites combined are presented below. Overall, method comparison study results satisfied the predetermined acceptance criteria for every hemoglobin fraction, demonstrating substantial equivalence between the candidate device and the predicate device.

Site 1 – Passing-Bablok Regression Results Summary							
Fraction	**N**	**Range**	**R**	**Slope**	**95% CI**	**Intercept**	**95% CI**
Hb A	122	8.7-97.2	0.999	1.007	1.000, 1.017	-0.74	-1.67, -0.20
Hb A2	179	1.6-6.1	0.995	1.000	1.000, 1.000	0	0.00, 0.00
Hb F	71	1.1-44.3	0.999	1.000	0.996, 1.003	0.10	0.06, 0.11
Hb S	70	19.7-76.8	0.998	0.953	0.941, 0.965	2.53	1.89, 3.04
Hb C	30	23.6-42.3	0.975	1.000	0.923, 1.114	0.85	-2.84, 3.30
Site 2 – Passing-Bablok Regression Results Summary							
Fraction	**N**	**Range**	**R**	**Slope**	**95% CI**	**Intercept**	**95% CI**
Hb A	119	6.8-97.2	0.999	0.973	0.954, 0.991	2.40	0.71, 4.17
Hb A2	128	1.8-5.9	0.958	1.000	1.000, 1.000	0.20	0.20, 0.20
Hb F	65	1.1-68.0	0.993	1.063	1.019, 1.091	0.82	0.74, 1.06

Site 1 – Passing-Bablok Regression Results Summary							
Fraction	**N**	**Range**	**R**	**Slope**	**95% CI**	**Intercept**	**95% CI**
Hb S	41	13.1-78.6	0.995	0.921	0.897, 0.952	1.09	-0.91, 2.53
Hb C	ND	ND	ND	ND	ND	ND	ND
Site 3 – Passing-Bablok Regression Results Summary							
Fraction	**N**	**Range**	**R**	**Slope**	**95% CI**	**Intercept**	**95% CI**
Hb A	79	10.5-97.2	0.998	0.973	0.949, 0.989	2.55	1.28, 4.74
Hb A2	105	1.9-6.9	0.931	0.963	0.893, 1.000	-0.09	-2.0, 0.17
Hb F	39	1.2-31.9	0.988	1.082	1.000, 1.149	0.86	0.57, 1.10
Hb S	32	9.6-73.9	0.995	0.908	0.864, 0.939	2.37	1.04, 3.91
Hb C	ND	ND	ND	ND	ND	ND	ND
All Sites Combined – Passing-Bablok Regression Results Summary							
Fraction	**N**	**Range**	**R**	**Slope**	**95% CI**	**Intercept**	**95% CI**
Hb A	320	6.8-97.2	0.999	0.990	0.984, 0.998	0.78	0.12, 1.40
Hb A2	412	1.6-6.9	0.957	1.000	1.000, 1.000	0.05	0.05, 0.05
Hb F	175	1.1-68.0	0.993	1.000	0.985, 1.020	0.70	0.52, 0.73
Hb S	143	9.6-78.6	0.994	0.929	0.909, 0.948	2.40	1.67, 3.22
Hb C	33	23.6-42.3	0.975	1.000	0.925, 1.103	0.60	-2.70, 3.06

2. Matrix Comparison:

Matrix studies comparing fresh, refrigerated, and frozen K2-EDTA anticoagulated venous whole blood samples were conducted to assess the effects of sample matrix on assay results. Hb A samples (normal and decreased due to phenotype), Hb A2 samples (normal and elevated), one Hb S sample (elevated), one Hb C samples (elevated) and 2 Hb F samples (elevated) were tested. The Hb F samples were only used in the refrigerated versus frozen comparison due to fresh sample availability, as Hb F samples were received from the vendor refrigerated. Where appropriate, samples were freshly drawn and an aliquot was frozen at <-70ºC overnight and another aliquot was stored at refrigerated temperatures overnight. On the following day, fresh samples were drawn again, and refrigerated/frozen samples were equilibrated to room temperature for comparison. Comparisons were fresh to refrigerated, fresh to frozen and refrigerated to frozen. Each Hb fraction level was analyzed 40 times over the course of 5 runs. Results were within predetermined acceptable bias for all Hb samples and conditions.

Passing-Bablok Regression Results				
Comparison	**Fraction**	**R**	**Slope (95% CI)**	**Intercept (95% CI)**
Fresh vs. Refrigerate	Hb A	0.999	1.008 (1.005, 1.011)	-0.75 (-1.06, -0.50)
	Hb A2	0.988	1.00 (1.00, 1.00)	0.00 (0.00, 0.00)
	Hb S	0.611	1.5 (1.00, 2.50)	-17.42 (-52.92, 0.20)
	Hb C	0.1934	1.00 (0.571, 2.333)	0.35 (-48.12, 15.93)
Fresh vs. Frozen	Hb A	0.999	1.010 (1.005,1.013)	-1.09 (-1.25, -0.63)
	Hb A2	0.986	1.00 (0.964, 1.00)	0.10 (0.10, 0.19)
	Hb S	0.250	-1.00 (-2.00, -0.333)	71.20 (47.57, 106.70)
	Hb C	0.049	-0.750 (-2.00, -0.25)	64.01 (45.88, 109.50)

Refrigerated vs. Frozen	Hb A	0.999	1.00 (0.998, 1.004)	0.00 (-0.33, 0.09)
	Hb A2	0.992	1.00 (0.996, 1.00)	0.00 (0.00, 0.18)
	Hb F	0.995	1.033 (1.016, 1.049)	-2.21 (-3.15, -1.26)
	Hb S	0.227	-0.500 (-1.00, -0.167)	53.67 (41.72, 71.60)
	Hb C	-0.0513	-0.987 (-1.436, -0.537)	-0.500 (-1.333, -0.200)

C Clinical Studies:

1. Clinical Sensitivity:

   Not Applicable

2. Clinical Specificity:

   Not Applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not Applicable

D Clinical Cut-Off:

Not Applicable

E Expected Values/Reference Range:

A reference range was established for Hemoglobin A and Hemoglobin A2 on the V8 Nexus Hemoglobin UltraScreen, using samples from 132 normal healthy individuals, studied across three sites. The resulting reference range for each hemoglobin fraction is as follows:

- Hb A: 96.6 – 97.9%
- Hb A2: 2.1 – 3.4%

The level of Hb F in normal population is below the level quantifiable by the V8 Nexus Hemoglobin UltraScreen assay. Thus, no reference interval was established for Hb F.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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