Laboratory Procedure Manual

Analyte: **Hemoglobin Variants**

Matrix: **Whole Blood**

Method: **Sebia Capillary 2 Flex Piercing Hemoglobin Electrophoresis Assay**

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As performed by: **Diabetes Diagnostic Laboratory University of Missouri School of Medicine 1 Hospital Dr. Columbia, MO 65212**

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Important Information for Users

The University of Missouri, Diabetes Diagnostic Laboratory periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
HGBVAR_K	LBXVC	Hemoglobin Variant C (%)
	LBXVD	Hemoglobin Variant D (%)
	LBXVE	Hemoglobin Variant E (%)
	LBXVF	Hemoglobin Variant F (%)
	LBXVS	Hemoglobin Variant S (%)
	LBXVU	Hemoglobin Variant U (%)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Hemoglobin is a complex molecule composed of two pairs of polypeptide chains. Each chain is linked to the heme, a tetrapyrrolic nucleus (porphyrin) which chelates an iron atom. The heme part is common to all hemoglobins and their variants. The type of hemoglobin is determined by the protein part called globin. Polypeptide chains α, β, δ and γ constitute the normal human hemoglobins:

- Hemoglobin A = α 2 β 2
- Hemoglobin A2 = α 2 δ 2
- Fetal hemoglobin F = α 2 γ 2

The α-chain is common to these three hemoglobins.

The hemoglobin spatial structure and other molecular properties (as that of all proteins) depend on the nature and the sequence of the amino acids constituting the chains. Substitution of amino acids by mutation is responsible for formation of hemoglobin variants which have different surface charges and consequently different electrophoretic mobilities, which also depend on the pH and ionic strength of the buffer.

The resulting qualitative (or structural) abnormalities are called hemoglobinopathies. Decreased synthesis of one of the hemoglobin chains leads to quantitative (or regulation) abnormalities, called thalassemias.

Hemoglobin electrophoresis is a well established technique routinely used in clinical laboratories for screening samples for hemoglobin abnormalities [1, 2, 3, 4, and 12]. The CAPILLARYS 2 FLEX PIERCING System has been developed to provide complete automation of this testing with fast separation and good resolution. In many respects, the methodology can be considered as an intermediary type of technique between classical zone electrophoresis and liquid chromatography [8, 11].

The CAPILLARYS 2 FLEX PIERCING System uses 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 [(5)].

The CAPILLARYS 2 FLEX PIERCING System has capillaries functioning in parallel allowing eight (8) simultaneous analyses for hemoglobin quantification from whole blood samples. A sample dilution with hemolyzing solution is prepared and injected by aspiration at the anodic end of the capillary. After high voltage protein separation, the hemoglobins are directly detected at 415 nm (wavelength specific for hemoglobin) at the cathode of the capillary. The resulting electrophoregrams are evaluated visually for pattern abnormalities.

By using alkaline pH buffer, normal and abnormal (or variant) hemoglobins are detected in the following order, from cathode to anode: δA'2 (A2 variant), C, A2/O-Arab, E, S, D, G-Philadelphia, F, A, Hope, Barts, J, N-Baltimore and H. Carbonic anhydrase is not visualized on the hemoglobin electrophoretic patterns. This permits identification of hemoglobin A2 variants in this migration zone. Direct detection provides accurate relative quantification of the individual hemoglobin fraction of interest, such as hemoglobin A2, diagnostic for β Thalassemia. In addition, the high resolution of this procedure allows for the identification of hemoglobin variants, particularly in the differentiation of hemoglobin S from D, and E from C. Hemoglobin A2 quantification can also be performed when hemoglobin E is present.

2. SAFETY PRECAUTIONS

Disconnect the line-power cord before doing any repairs, hardware troubleshooting or preventative maintenance.

All healthcare personnel shall routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood or other body fluids of an patient is anticipated. All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious disease. Wear appropriate Personal Protective Equipment (PPE), including facial protection, gloves, and protective clothing. Wash hands thoroughly after handling specimens and kit reagents.

Dispose of all biological samples and diluted specimens in a biohazard waste container at the end of analysis. Dispose of all liquid hazardous waste in proper hazardous waste container.

Handle all patient specimens, controls, and reagents using Universal Precautions. This includes specimens diluted for analysis. No test method can offer complete assurance that Hepatitis B (and C) Virus, Human Immunodeficiency Virus (HIV) or other infectious agents are absent. Therefore, all human blood products (including controls) as a hazardous biological material and should be considered potentially infectious. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation where it exists, for the USA: Center for Disease Control/National Institutes manual "Biosafety in Microbiological and Biomedical Laboratories", 1984.

Refer to the MSDS for each reagent for specific precautions.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Data are maintained on a secured Microsoft Access/ Microsoft SQL server client-server system in a 128-bit authenticated Windows domain environment.

1. Laboratory services are requested through the Westat system operations via an email notification containing a unique manifest list of the samples

and sample analysis type (e.g. GHB), which confirms that specimens have been shipped to DDL.

2. Each Manifest Form should include and be verified against each sample received:
   a. Patient Sample ID #
   b. Test Name
   c. Date Collected
   d. Shipment ID #
   e. Shipment Date
   f. Lab Name
   g. Lab ID
   h. Survey Year
3. Once specimens are received and verified the corresponding file is imported electronically into the SQL server database via secure transfer.
4. After analysis the results, date analyzed and tech initials are imported from the instrument into the SQL server database via secure transfer.
5. Data check sheets are printed out and checked against the instrument printouts by the supervisor or delegate.
6. After results are cleared by the supervisor a results file in the specified format is exported and uploaded to Westat via secure transfer.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

i. Specimen Collection and Handling

Each specimen should be labeled with a sample ID and test name. Sample IDs should be verified against the sample manifest that list the below;

a. Patient Sample ID #
b. Test Name
c. Date Collected

d. Shipment ID #

e. Lab Name

ii. Sample collection and storage.

1. Fresh anticoagulated whole blood samples collected in tubes containing K_2EDTA or K_3EDTA as an anti-coagulant are recommended for analysis.
2. Samples may be stored for up to seven (7) days at 2-8°C. Note: Samples should not be stored at room temperature (15 – 30 °C)!

   When the blood sample is stored for more than 7 days at 2-8°C:

   a. A weak fraction, corresponding to methemoglobin, appears in the Hb S migration zone;
   b. When Hb C is present, a fraction corresponding to degraded Hb C appears more anodic than Hb A2 which does not interfere with it (Z(E) zone, see the table in paragraph “Interpretation”);
   c. When Hb O-Arab is present, a fraction corresponding to degraded Hb O-Arab appears in the Hb S migration zone (Z(S) zone, see the table in paragraph “Interpretation”);
   d. When Hb E is present, a fraction corresponding to degraded Hb E appears in the Z(D) zone (see the table in paragraph “Interpretation”);
   e. When Hb S is present, a fraction corresponding to degraded Hb S appears in the Hb F migration zone (Z(F) zone, see the table in paragraph “Interpretation”);
   f. When Hb A is present, a fraction corresponding to degraded Hb A (“aging fraction” of Hb A) appears more anodic (Z11 zone, see the table in paragraph “Interpretation”);
   g. When stored for more than (ten) 10 days, viscous aggregates in red blood cells (clots may form) are observed; they must be removed before the analysis.

iii. Sample Preparation

Analysis of samples with volumes less than 1 mL:

1. Vortex the whole blood sample for five seconds.
2. Apply 100µL of whole blood (to be analyzed) into a conical tube for control and cap the tube.
3. Place the tube with a wedge adapter on a sample rack of the CAPILLARYS 2 FLEX PIERCING system.
4. Slide the sample rack into the CAPILLARYS 2 FLEX PIERCING system.
5. Perform the analysis of this sample according to the standard procedure just as a typical blood sample.

NOTE: The sample cannot be identified without a barcode label.

Particular Cases

Analysis of samples without any Hb A or Hb A2 (these samples are quantified ,but not identified by zones):

To identify hemoglobin fractions in a sample without any Hb A or Hb A2, it is recommended to prepare a sample for re-analysis according to the following procedure:

1. Vortex the whole blood sample for 5 seconds.
2. In a conical tube for control, mix one volume (50µL) of whole blood to be analysed with one volume (50µL) of Normal Hb A2 Control and cap the tube.
3. Vortex the mixture for 5 seconds.
4. Place the tube with a wedge adapter on a sample rack of the CAPILLARYS 2 FLEX PIERCING system.

5. Slide the sample rack into the CAPILLARYS 2 FLEX PIERCING system.
6. Perform the analysis of this sample according to the standard procedure just as a typical blood sample.

The results are then automatically recognized by the software for data analysis.

IMPORTANT: For a sample without any Hb A or Hb A2, prepared according to the above procedure, the result obtained with the mixed sample will enable presumptive variant identification due to the positioning of the hemoglobin fractions in the appropriate identification zones. DO NOT REPORT THE RELATIVE QUANTIFICATION FROM THE MIXED SAMPLE RESULT. The relative quantification of the hemoglobins should be reported using the initial, unmixed sample result (without any dilution in the blood control).

Re-analysis of a sample with an additional fraction in the Z(C) migration zone (Hb C migration zone) or Z(A2) migration zone (Hb A2 migration zone):

The presence of a Hb Constant Spring variant may be suspected when a hemoglobin fraction is observed in Z(C) or Z(A2) migration zones. This fraction may also be due to plasma proteins in the sample (decreased hematocrit as in a patient with anemia), for example. An analysis of red blood cells from the same sample, without plasma proteins, will confirm the presence of this variant. Prepare the sample according to the following procedure:

1. Centrifuge the whole blood sample at 5,000 rpm for 5 minutes; discard the plasma.
2. In a microtube, mix one volume (50µL) of red blood cells from the sample to analyze with eight volumes (400µL) of CAPILLARYS HEMOGLOBIN(E) hemolyzing solution.

3. Vortex for five seconds.
4. Add 100µL the prepared hemolysate into the wells of another green dilution segment.
5. Place this dilution segment on the “Zero” (No. 0) sample rack of CAPILLARYS 2 FLEX PIERCING.
6. Slide the sample rack into the CAPILLARYS 2 FLEX PIERCING system and select “sample” with “manual dilution” in the window which appears on the screen and validate.

The results are then automatically recognized by the software for data analysis.

iv. Unacceptable Specimen Criteria

1. Avoid coagulated blood specimens.
2. Avoid aged and/or improperly stored blood samples since the automated hemolysis of samples may be impaired by clots and these degradation products (as artifacts) may affect the electrophoretic pattern.
3. Reject specimens collected in preservatives/anticoagulants other than EDTA tubes.
4. Avoid specimens which have leaked their contents (document the specimen IDs of leaky specimens and contact NHANES immediately).
5. Only whole blood specimens are acceptable.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

1. Reagents and Materials Supplied in the Kit

Buffer (ready to use)	2 vials, 700 mL each
Hemolyzing solution (ready to use)	1 vial, 440 Ml
Wash solution (stock solution)	1 vial, 75 Ml
Dilution segments	1 pack of 90
Filters	4 filters

FOR OPTIMAL RESULTS: All reagents from the same kit must always be used together and according to the package insert instructions.

WARNING: Do not use marketed deionized water, such as water for ironing, for example, (risk of significant capillary damage). Use only water with ultrapure quality, such as injection grade water (or Type I water)

a. Buffer

The buffer is ready to use. It contains: alkaline buffer pH 9.4 and additives, nonhazardous at concentrations used, necessary for optimum performance.

Store the buffer refrigerated (2-8°C). It is stable until the expiration date indicated on the kit package or buffer vial labels. Avoid storage at room temperature for a long time or close to a window or to a heat source. DO NOT FREEZE.

IMPORTANT: Prior to use, when stored at 2-8°C, it is necessary for the buffer to reach room temperature. When it is full, let the buffer vial set at room temperature for at least three (3) hours prior to use. If this precaution is not respected, the performance of the procedure may be affected. ***WARNING: Do not pre-heat the buffer in hot water.***

Once the buffer vial has been opened and positioned on the CAPILLARYS 2 FLEX PIERCING system, it is stable for a maximum of **ONE MONTH**

(accumulated) at room temperature (15-30°C). After each use, it is important that the buffer be stored refrigerated (2-8°C) immediately; it is then stable until the expiration date on the buffer vial label.

IMPORTANT: The accumulated time of the buffer stored at room temperature must not exceed **ONE MONTH**. This time of ONE MONTH storage takes account of the time for the buffer to come to room temperature.

Discard buffer if its appearance changes, especially if it becomes cloudy due to microbial contamination.

b. Hemolyzing Solution

Hemolyzing Solution is ready to use. It is a buffer pH 8.5 ± 0.5 with additives, nonhazardous at the concentration used, necessary for optimum performance.

It is used to dilute and hemolyze red blood cells.

Store Hemolyzing Solution at room temperature (15-30°C) or refrigerated (2-8°C). It is stable until the expiration date indicated on the kit package or Hemolyzing Solution vial label. DO NOT FREEZE.

Discard Hemolyzing Solution if its appearance changes, especially if it becomes cloudy due to microbial contamination.

c. Wash Solution

The vial of the stock wash solution should be diluted up to 750 mL with distilled or deionized water. After dilution, the wash solution contains an alkaline solution, pH ≈ 12. Wash solution is used for washing the capillaries before and after hemoglobin electrophoresis.

WARNING: The wash solution contains sodium hydroxide. Corrosive solution. Causes severe burns. Keep out of reach of children. In case of

contact with eyes, rinse immediately with plenty of water and seek medical advice. Immediately take off all contaminated clothing. Wear suitable clothes and eye/face protection.

Store the stock and working wash solutions in closed containers at room temperature or refrigerated. The stock wash solution is stable until the expiration date indicated on the kit or wash solution vial label. Working wash solution is stable for three (3) months.

Discard Working Wash Solution if its appearance changes, especially if it becomes cloudy due to microbial contamination.

d. Dilution Segments

Use

Colored single use segments for blood sample dilution on the automated instrument. They are specific for CAPILLARYS HEMOGLOBIN(E) procedure.

WARNING: Dilution segments containing biohazardous samples must be handled with care.

e. Filters

Use

Disposable filters for filtration of analysis buffer, hemolyzing solution (for the CAPILLARYS 2 FLEX PIERCING system) working wash solution, and distilled water (used for capillary rinsing).

IMPORTANT: With each new kit, always change all the four filters.

Screw one filter at the connector situated at the end of each tube that plunges in the vials of buffer, hemolyzing solution, wash solution and distilled or deionized water. When placing filters on the CAPILLARYS

system, rinse the connectors and the tubes with distilled or deionized water. Used filters must be rinsed before being discarded.

The filter intended for analysis buffer must be used for filtration of both buffer vials; the two other filters are intended for filtration of hemolyzing solution, working wash solution, and for distilled or deionized water (for capillary rinsing).

Before use, store the filters in their sealed package in a dry place at room temperature (15-30°C) or refrigerated (2-8°C).

2. Reagents Required but not supplied

- NORMAL Hb A2 CONTROL
  The Normal Hb A2 Control is obtained from a pool of normal human blood samples. It contains normal levels of Hb A and HbA2.

- THE PATHOLOGICAL Hb A2 Control
  The Pathological Hb A2 Control is obtained from a pool of human blood samples with an increased hemoglobin A2 fraction.
- The Hb AFSC Control is obtained from a pool of normal blood samples with normal hemoglobins A and F and abnormal hemoglobins S and C (contains at least three known major hemoglobins including both a sickling and a nonsickling hemoglobin).
  Refer to the Instructions for Use/Package Inserts for processing, handling, and storage details for each control level.

- Distilled or Deionized Water
  Used for rinsing the electrophoresis capillaries run on the CAPILLARYS 2 FLEX PIERCING, SEBIA automated system. It is recommended to use water/deionized water filtered over a ≤0.45 porosity filter and having a resistivity greater than 10 Megaohms x cm. Change the water daily to avoid

microbial growth. For longer storage, add 35 µL/dL of CLEAN PROTECT (SEBIA, PN 2059, 5mL).

IMPORTANT: Before filling the rinse container, it is recommended to wash it with plenty of distilled or deionized water.

- CAPICLEAN

Composition

The vial of CAPICLEAN concentrated solution (SEBIA, PN 2058, 25mL) contains proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, and necessary for optimum performance.

WARNING: The CAPICLEAN solution may cause irritation or burns to skin, eyes and mucous membranes.

Use

For weekly cleaning of capillaries and sample probe in the CAPILLARYS 2 FLEX PIERCING, SEBIA automated system for capillary electrophoresis.

IMPORTANT: Launch a CAPICLEAN cleaning sequence once a week if running less than 500 analyses in a week, or after every 500 analyses if performed within less than a week, or daily if more than 500 analyses are run in one day. (*See the CAPICLEAN, SEBIA, package insert instructions.)*

IMPORTANT: Do not re-use the dilution segment after sample probe cleaning.

Storage, stability and signs of deterioration

Store CAPICLEAN refrigerated (2-8°C). It is stable until the expiration date indicated on the vial label. DO NOT FREEZE.

A precipitate may be observed in the CAPICLEAN vial without any adverse effects on its utilization. Do not dissolve the precipitate. It is recommended to collect only the supernatant.

- SODIUM HYPOCHLORITE SOLUTION (for sample probe cleaning)

Preparation

Prepare a sodium hypochlorite solution (2% to 3% chloride) by diluting 250mL 9.6% chloride solution to 1 liter with cold distilled or deionized water.

Use

For sample probe cleaning in the CAPILLARYS 2 FLEX PIERCING system (weekly maintenance in order to remove proteins adsorbed on the probe).

See the instruction sheets of CAPILLARYS 2 FLEX PIERCING, SEBIA.

- Use the sample rack designed for the maintenance (No. 100).
- Place a tube containing 2mL diluted chlorinated solution previously prepared, in position No. 1 on this sample rack.
- Slide the sample rack No. 100 for maintenance in the CAPILLARYS 2 FLEX PIERCING system.
- In the "MAINTENANCE" window which appears on the screen, select "Launch Probe Cleaning (chlorinated sodium hypochlorite solution)" and validate.

Storage, stability and signs of deterioration

Store the working chlorinated solution at room temperature in a closed container; it is stable for three (3) months. Avoid storage in sunlight, close to heat and ignition source, or close to acids and ammonia.

- CAPILLARYS/MINICAP WASH SOLUTION

Preparation

Each vial of the stock Wash Solution (SEBIA, PN 2052, 2 vials, 75mL) should be diluted up to 750mL with distilled or deionized water. After dilution, the wash solution contains an alkaline solution, pH ≈ 12.

WARNING: The wash solution contains sodium hydroxide. Corrosive solution. Causes severe burns. Keep out of reach of children. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Take off all contaminated clothing immediately. Wear suitable clothes and eye/face protection.

Use

For washing the capillaries of CAPILLARYS 2 FLEX PIERCING. This additional reagent is needed when the number of tests in a series is less than 40.

Storage, stability and signs of deterioration

Store the stock and working wash solutions in closed containers at room temperature or refrigerated. The stock wash solution is stable until the expiration date indicated on the kit or wash solution vial label. Working wash solution is stable for three (3) months. Discard working wash solution if its appearance changes, especially if it becomes cloudy due to microbial contamination.

NOTE: The assays that were performed for the validation of reagents demonstrated that, for the different solutions while using automated pipettors, a variation of ±5% on the final volume had no adverse effect on the analysis. The distilled or deionized water used to reconstitute solutions, must be free of microbial growth (filter using a ≤ 0.45 filter) and have a resistivity higher than 10 Megohms x cm.

3. EQUIPMENT AND ACCESSORIES REQUIRED

a. CAPILLARYS 2 FLEX PIERCING System SEBIA, PN 1227.

b. Sample racks supplied with CAPILLARYS 2 FLEX PIERCING.
c. Container Kit supplied with CAPILLARYS 2 FLEX PIERCING: Rinse (fill with distilled or deionized water), wash solution and waste container.
d. Tubes and caps for Controls, SEBIA, PN 9205: 500 conical tubes and their caps to analyze blood controls with the CAPILLARYS 2 FLEX PIERCING system.
e. Wedge adapters for tubes for controls (yellow), SEBIA, PN 9203, 10 units (or supplied with CAPILLARYS 2 FLEX PIERCING).

4. PRODUCT STORAGE AND SAFETY

1. Buffer

   Store the buffer refrigerated (2-8°C). It is stable until the expiration date indicated on the kit package or buffer vial labels. Avoid storage at room temperature for a long time or close to a window or to a heat source. DO NOT FREEZE.

   IMPORTANT: Prior to use, when stored at 2-8°C, allow the buffer to reach room temperature. When it is full, let the buffer vial set at room temperature for at least three (3) hours prior to use. If this precaution is not respected, the performance of the procedure may be affected. ***WARNING: Do not pre-heat the buffer in hot water.***

   Once the buffer vial has been opened and positioned on the CAPILLARYS 2 FLEX PIERCING system, it is stable for a maximum of **ONE MONTH** (accumulated) at room temperature (15-30°C). After each use, it is important that the buffer be stored refrigerated (2-8°C) without delay; it is then stable until the expiration date on the buffer vial label.

   IMPORTANT: The accumulated time of the buffer stored at room temperature must not exceed **ONE MONTH**. This time of ONE MONTH storage includes the time for the buffer to come to room temperature.

Discard buffer if its appearance changes, especially if it becomes cloudy due to microbial contamination.

2. Hemolyzing Solution

   Store Hemolyzing Solution at room temperature (15-30°C) or refrigerated (2-8°C). It is stable until the expiration date indicated on the kit package or Hemolyzing Solution vial label. DO NOT FREEZE.

   Discard Hemolyzing Solution if its appearance changes, especially if it becomes cloudy due to microbial contamination.

3. Wash Solution

   Store the stock and working wash solutions in closed containers at room temperature (15-30°C) or refrigerated (2-8°C). The stock wash solution is stable until the expiration date indicated on the kit or wash solution vial label. Working wash solution is stable for three months. Discard Working Wash Solution if its appearance changes, especially if it becomes cloudy due to microbial contamination.

4. CAPICLEAN

   Store CAPICLEAN refrigerated (2-8°C). It is stable until the expiration date indicated on the vial label. DO NOT FREEZE.

   CAPICLEAN must be free of precipitate. Discard CAPICLEAN if its appearance changes, especially if it becomes cloudy due to microbial contamination

   WARNING: The CAPICLEAN solution may cause irritation or burns to skin, eyes and mucous membranes.

5. Sodium Hypochlorite Solution

   Store the working chlorinated solution at room temperature in a closed container. It is then stable for three (3) months. Avoid storage in sunlight, close to heat and ignition source, and or close to acids and ammonia.

Refer to the Instructions for Use/Package Inserts for additional details on each reagent.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Not applicable for this procedure

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

The CAPILLARYS 2 FLEX PIERCING system is a multi-parameter instrument for hemoglobin analysis on parallel capillaries. The hemoglobin assay uses eight (8) capillaries to run the samples.

The sequence of automated steps is as follows:

- Bar code reading of sample tubes (for up to 8 tubes) and samples racks;
- Mixing of blood samples before analysis;
- Sample hemolysis (lysis) 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.

The manual steps include:

- Placement of sample tubes (with caps) in sample-racks in positions 1 to 8;
- Placement of new dilution segments in sample-racks;
- Placement of racks on the CAPILLARYS 2 FLEX PIERCING instrument;
- Removal of sample-racks after analysis.

1. Preparation for Capillarys Analysis

PLEASE READ THE CAPILLARYS 2 FLEX PIERCING INSTRUCTION MANUAL CAREFULLY.

a. Switch on the CAPILLARYS 2 FLEX PIERCING instrument and computer.
NOTE: The instrument must be switched on BEFORE the computer so that the firmware is recognized by the computer software upon start-up.

b. Start up the software, enter and the instrument automatically will start.

c. The CAPILLARYS HEMOGLOBIN(E) kit is intended to run with the HEMOGLOBIN(E) analysis program on the CAPILLARYS 2 FLEX PIERCING instrument. Select the "HEMOGLOBIN(E)" analysis program and place the CAPILLARYS HEMOGLOBIN(E) buffer vial in the instrument. Please read the CAPILLARYS 2 FLEX PIERCING instruction manual carefully.

- The sample rack contains eight (8) positions for sample tubes. Place up to eight (8) capped sample tubes with whole blood on each sample rack in positions 1-8. The barcode of each tube must be visible in the openings of the sample rack.
  IMPORTANT: If the number of tubes to analyze is less than eight, complete the sample rack with capped tubes containing distilled or deionized water.

- Position a new dilution segment on each sample rack. The rack will be rejected if the segment is missing.
- Slide the complete sample racks into the CAPILLARYS 2 FLEX PIERCING System through the opening in the middle of the instrument. Up to 13 sample racks can be introduced successively and continuously into the system. **Sample rack “zero” (No. 0) is intended for control blood samples with specific tubes, caps and the wedge adapter designed for the control tubes.**

- Remove analyzed sample racks from the plate on the left side of the instrument.

- Remove used dilution segments from the sample rack and discard them. **WARNING: Dilution segments with biological samples are biohazardous and must be handled with care.**

DILUTION - MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

- Barcodes are read on both sample tubes and sample racks.
- Mixing of tubes.
- Samples are diluted in hemolyzing solution and the sample probe is rinsed after each sample.
- Capillaries are washed.
- Diluted samples are injected into capillaries.
- Migration is carried out under constant voltage for about 8 minutes and the temperature is controlled by the Peltier effect.
- Hemoglobin fractions are detected directly by scanning at 415 nm and an electrophoretic profile appears on the screen of the system.

NOTE: *These automated steps described above are applied to the first in sample rack inserted. The electrophoretic patterns appear after about 20 minutes from the start of the analysis. For the following sample rack(s), the first three steps (barcode reading, mixing, and sample dilution) are performed during analysis of the previous sample rack.*

2. Result Analysis

At the end of the analysis, relative quantification of the individual hemoglobin fractions occurs automatically and their profiles can be analyzed. Hemoglobin fractions Hb A, Hb F and Hb A2 are automatically identified and the Hb A fraction

is adjusted to the middle of the review window. The resulting electrophoregrams are evaluated visually for pattern abnormalities.

The potential positions of the different hemoglobin variants (identified by zones Z1 to Z15) are shown on the screen of the system and are indicated on the result strip. The name of the zone is framed by name when the software identifies a hemoglobin fraction in a defined zone.

Patterns are automatically adjusted with respect to the Hb A and Hb A2 fractions to facilitate their interpretation:

- When Hb A and/or Hb A2 fractions are not detected on an electrophoretic pattern, a yellow warning signal appears and an adjustment is made using the position of the Hb A fraction on the previous two patterns obtained on that same capillary. Then, there are no fractions identified, except when Hb C is detected, in this case, Hb A2 and Hb C fractions are identified.
- When Hb F is detected on an electrophoretic pattern, without detection of Hb A, the yellow warning signal does not appear. The adjustment is then made using the position of the Hb F fraction and the Hb F and/or Hb A and/or Hb A2 fractions are identified.
- When the adjustment is not possible, a red warning signal appears, Hb F and Hb A2 fractions are then not identified (Call SEBIA).
- When the optical density (OD) is insufficient on a migration control electrophoretic pattern (obtained with the Normal Hb A2 Control, identified with its barcode label on the sample rack No. 0), a warning message is displayed in order to consider removing this control from analysis for the determination of Hb A fraction position. Then, a purple warning signal appears on the review window and Hb A and Hb A2 fractions are not identified.

The different variant zones (Z1 to Z15) do not appear on the screen of the system or on the result strip. But on the electrophoretic pattern, the Hb A2 and Hb C fractions are calculated and re-drawn with adjustment (fitted) and are on

the original curve. This display then allows the quantification of Hb A2 if Hb C is present in the sample. On the electrophoretic pattern, the curves of Hb A2 and Hb C fractions are calculated and redrawn by fitting with adjustment (or fitted) and are overlaid on the original curve. This display allows the quantification of Hb A2 if Hb C is present in the sample.

WARNING: In some cases of Hb C (homozygous) or after a technical problem, the Hb A2 and Hb C curves are shifted and incorrectly integrated (not fitted properly). These fractions are then under-quantified. It is then recommended to quantify the Hb A2 fraction using another technique.

PLEASE READ THE CAPILLARYS FLEX PIERCING INSTRUCTION MANUAL CAREFULLY.

3. End of Analysis Sequence

At the end of each analysis sequence, the operator must initiate the "Shut Down" procedure of the CAPILLARYS 2 FLEX PIERCING system to store the capillaries under optimal conditions.

4. Filling Reagent Containers

The CAPILLARYS 2 FLEX PIERCING system has an automatic reagent control.

IMPORTANT: Before filling the rinse container, it is recommended to wash it with plenty of distilled or deionized water.

WARNING: Do not use marketed deionized water, such as water for ironing (risk of significant capillary damage). Use only water with ultrapure quality, such as injection grade water.

IMPORTANT: Please refer to the instructions for replacement of reagent containers, respecting color codes for vials and connectors.

A message will be displayed when it is necessary to perform one of the following tasks:

- Place a new buffer container;
- Place a new hemolyzing solution container;
- Fill the container with working wash solution;
- Fill the container with filtered distilled or deionized water for rinsing capillaries;
- Empty the waste container.

PLEASE READ THE CAPILLARYS FLEX PIERCING INSTRUCTION MANUAL CAREFULLY.

INTERPRTATION

The different migration zones for hemoglobin variants designated Z1 to Z15) are shown on the screen of the system and on the result strip. Passing the mouse cursor over a zone name will display information containing hemoglobin variants possibly seen in this zone. For each fraction, the maximum position defines the migration zone.
See the table showing the potential variants in each of the various zones.

Note: Sebia does not advise use of any of the “curve edit” tools (including the “manual baseline,” “change baseline slope,” “delete artifact,” “delete zone,” or “smoothing” tools) on hemoglobin results. The instrument should not be displaying elevated baseline on anything other than severely degraded samples, and the appearance of a baseline may indicate a need to service the instrument. The presence of small apparent artifactual on the hemoglobin pattern should be similarly viewed as possible indication that the instrument needs service, or the presence of an Hb A2 variant (delta or alpha chain variant hemoglobin resulting in a subdivision of the Hb A2). By default, the system has “smoothing” set to the lowest setting. Increasing smoothing may be detrimental to peak separation.
g. The use of the “zoom” tool is advisable to look closely at small fractions. The use of the “overlay reference pattern” tool is useful to assist in variant identification when applying other reference hemoglobin patterns (AFSC control, etc.) over a

current pattern which has properly zoned. Do not use if the current pattern has not "zoned." See attached email communication document from Sebia regulatory department.

WARNING: Extension of the horizontal axis does not, in any case, allow for the identification of a hemoglobin variant.

1. *Qualitative abnormalities: Hemoglobinopathies*

   Most hemoglobinopathies are due to substitution by mutation of a single amino acid in one of the four types of polypeptide chains [1, 2, 4, 9, and 12]. The clinical significance of such a change depends on the type of amino acid and the site involved [13]. In clinically significant disease states, either the α-chain or the β-chain is affected.

   More than 1400 variants of adult hemoglobin have been described [6, 14]. The first abnormal hemoglobins studied and the most frequently occurring have an altered net electric charge, leading to easy detection by electrophoresis.

   There are five (5) main abnormal hemoglobin fractions are of particular clinical interest: S, C, E, O-Arab and D.

   The CAPILLARYS HEMOGLOBIN(E) kit is intended for the identification of hemoglobinopathies and thalassemias.

   Hemoglobin S

   Hemoglobin S is the most frequent hemoglobinopathy. It is the result of the substitution of glutamic acid by neutral amino acid valine on the β-chain at position 6. When compared against Hb A, its isoelectric point is elevated and its total negative charge decreased at the pH for the analysis. Its electrophoretic mobility is, therefore, increased in the capillary and this hemoglobin is faster than the A

fraction. Hemoglobin S migrates between the Hb A and Hb A2 fractions, next to Hb A2 with the alkaline buffered CAPILLARYS HEMOGLOBIN(E) procedure.

Hemoglobin C

In Hemoglobin C, glutamic acid is replaced by basic amino acid lysine on the ß-chain at position 6 and its mobility is strongly reduced. When compared to Hb A, its isoelectric point is highly elevated and its total negative charge decreased at the pH of analysis. Its electrophoretic mobility is, therefore, increased in the capillary and this hemoglobin is faster than the Hb A fraction allowing for its differentiation. Hemoglobins C, E and O-Arab are not superimposed on the electrophoretic pattern and are easily identified.

Hemoglobin E

In Hemoglobin E, glutamic acid is replaced by basic amino lysine on the β-chain at position 26. With the CAPILLARYS HEMOGLOBIN(E) procedure, hemoglobin E migrates just anodic behind hemoglobin A2 and is totally separated from it. When hemoglobin E is present, the Hb A2 fraction can be determined to detect β thalassemia.

Hemoglobin O-Arab

In Hemoglobin Arab, glutamic acid is replaced by basic amino acid lysine on the β-chain at position 121. With the CAPILLARYS HEMOGLOBIN(E) procedure, hemoglobin O-Arab migrates exactly as hemoglobin A2. In this case, hemoglobin A2 can not be quantified. When the Hb A2 fraction is **> 9%,** hemoglobin O-Arab must be suspected. Note that Hb O-Arab migrates separately from hemoglobins C and E.

Hemoglobin D (-Los Angeles)

In Hemoglobin Los Angeles, glutamic acid is replaced by glutamine on the β-chain at position 121. With the CAPILLARYS HEMOGLOBIN(E) procedure, hemoglobin

D (called D-Punjab, D-Los Angeles, D-Chicago or D-Portugal) migrates behind hemoglobin S. This property allows for differentiation of Hb S and Hb D.

2. *Quantitative abnormalities: Thalassemia*

Thalassemias constitute a quite heterogeneous group of genetic disorders characterized by decreased synthesis of one type of the polypeptide chains. The molecular mechanism of this decrease has not been fully described.

There are two types of thalassemia syndromes:

Alpha-thalassemia

Alpha-Thalassemia is characterized by the decreased synthesis of the α-chains, thus, affecting the synthesis of the normal hemoglobins.

Excessive synthesis of β- and γ-chains in relation to that for the α-chains results in the formation of tetramers devoid of α-chains:

- hemoglobin Bart = γ 4
- hemoglobin H = β 4.

Hemoglobin H has a low isoelectric point. When using the CAPILLARYS HEMOGLOBIN(E) procedure, it migrates more anodic than hemoglobin A and may appear as one or several fractions.

Beta-thalassemia

Beta-Thalassemia is characterized by the decreased synthesis of the β-chain, where hemoglobin F and hemoglobin A2 percentages are increased with respect to the hemoglobin A and β chain variants.

Detection of beta-thalassemia is possible through comparison against the values obtained for normal hemoglobin fractions using the CAPILLARYS HEMOGLOBIN(E) procedure.

3. Particular cases

- When there is no hemoglobin A in the sample, a small fraction may be observed in its migration zone; this fraction may be acetylated hemoglobin F which represents about 15 to 25% of hemoglobin F. The CAPILLARYS 2 FLEX PIERCING system can easily identify this acetylated hemoglobin separately from the hemoglobin A.

  A hemoglobin A2 variant may be suspected when a small fraction (about 0.5 to 3%) migrates between hemoglobins F and δA'2 (A2 variant),
- When a hemoglobin A2 variant is detected (δA'2 or any other A2 variant), it is recommended to add its percentage to that for hemoglobin A2 to better diagnose beta-thalassemia.
- Some hemoglobin variants, such as Hb Camperdown and Hb Okayma, migrate close to Hb A and may not be separated from this hemoglobin.
- Some hemoglobin variants, such as Hb Porto-Alegre and degraded Hb S, migrate close to Hb F and may not be separated from it.
- Weak hemoglobin fractions migrating in zone Z12, particularly Hb Barts, are sometimes inaccurately quantified. In this case, delete the automatic quantification and then quantify the fractions manually.
- Weak fractions may be observed in the Z14 and Z15 migration zones. In this case, review the hematologic status of the patient and do additional testing to distinguish these fractions as either an artifact or an actual hemoglobin abnormality.
- When analyzing blood samples from transfused patients with sickle cell disease, with a low Hb A level (less than 10%), the Hb S fraction may appear shifted from the Z5 zone to the Z6 zone. In this case, review the hematological status of the patient and to perform additional testing to confirm the presence of Hb S.
- When analyzing blood samples from newborn babies, samples containing high levels of Hb F may affect %Hb A, particularly from the presence of degraded Hb F in its migration zone. Here, the Hb A percentage indicated by the software may be overvalued. Additionally, when hemoglobin variants (> 4), such as Hb S, Hb C, Hb

E or Hb D-Punjab, are present in blood samples containing high Hb F at levels (> 60%), additional testing is necessary to confirm the Hb A concentration.

- For newborn babies, between 6-9 months old, it is recommended to collect and analyze multiple blood samples, such as monthly, to monitor the Hb F concentration. This will allow for verification of the decrease of the Hb F concentration and the potential presence of a variant. For cases in question, it is recommended to confirm by additional studies including analysis of the parents' blood.

- Examples with increased Hb F (except for newborn babies)
    - Pregnancy
    - Patients with sickle cell disease, more than 2 years old, with hydroxyurea treatment and/or transfused and/or naturally producing Hb F increased by compensation
    - Patients, two (2) years old and older, with hereditary persistence of fetal hemoglobin (HPFH problems) showing 20 to 40% Hb F in heterozygous patients.
    - Patients, older than two years, with any type of leukemia, hereditary hemolytic anemia, diabetes, thyroid disease, bone marrow hyperplasia, multiple myeloma, cancer with metastases.

      For further information, please refer to http://www.answers.com/topic/fetal-hemoglobin-test

REPORTING RESULTS: PROCEDURE

1. In the Sebia Phoresis software, review each electropherogram and record the variant and position number in the Comments field.
2. Print the electropherograms and run list. To print the result list, make sure the correct run date and method (Hemoglobin (e)) are selected. On the menu bar select Edit Curve, then Result List, then Print. This will generate a

new table, click Print again to print the table. Close out of the tables. Click Print on the menu bar to print out the electropherograms.

3. Review the control results and verify that they are acceptable.
4. In the NHANES database, select “Enter Results: then “Enter Sebia Variants” to display the variant results entry screen.
5. For each corresponding sample ID, enter the run date, tech initials, select the variant type (HbC, HbD, HbE, HbF, HbS or Hbx if unknown) and the % of the variant. If more than one variant is present, additional corresponding fields for each variant should be entered under the same sample ID by clicking the + symbol next to the run date. Click Save after entering each record.
6. After all results have been entered click Close. The data entry check sheet will print automatically.
7. Submit check sheet(s), electropherograms and result list printouts to supervisor for verification.
8. Supervisor will verify acceptability of entire run based upon controls, and acceptability of individual results based upon the electropherograms.
9. When satisfied, the supervisor will clear the results in the NHANES database.
10. Reporting Format: Results are expressed on the report as % of the variant and are rounded to one decimal place. Results are reported throughout the entire range of variant values.

Refer to the Appendix section for the overall workflow flow chart scheme and the NHANES Study Handling and Reporting Procedure in Navex; G8 HbA1c analysis to Hb Electrophoresis reporting, with designated comment codes.

9. REPORTABLE RANGE OF RESULTS

This assay is a semi-quantitative. For the purposes of this **NHANES study 2019-2020** Cycle presumptive hemoglobin variants S, C, D, and E (abnormal variant observed), and elevated HbF (over 2%), initially screened by the G8 HPLC method and reflexed to the Capillarys 2 Flex Piercing will be reported as percentages.

10. QUALITY CONTROL (QC) PROCEDURE

1. The Normal Hb A2 Control – The Normal Hb A2 Control is used:

- for the migration control of the human hemoglobin pattern and,
- for the quantitative quality control for human hemoglobins A and A2,

with the SEBIA electrophoresis CAPILLARYS HEMOGLOBIN(E) used with the CAPILLARYS 2 FLEX-PIERCING. The Normal Hb A2 Control is designed for laboratory use. It should be used (with its bar code label for the CAPILLARYS) like a normal human blood. The values obtained must fall within the range provided with each batch of Normal Hb A2 Control.

i. The Normal Hb A2 control should be treated as normal human blood.

ii. QC specimens are tested in the same manner as patient specimens and by the same personnel performing patient testing.

iii. QC should be handed and stored according to manufacturers' guidelines.

iv. After reconstitution (with the volume indicated on control package insert), use the Normal Hb A2 Control directly as a blood sample to be analyzed or as a migration control (added to a tube for the control with its cap, identified with a barcode label, and analyzed with the sample rack "zero" (No. 0) using the wedge adapter, as described above). It will be diluted automatically with hemolyzing solution.

Refer to the Instructions For Use (IFU) Normal Hb A2 Control instructions, attached in this document for reconstitution, storage, and handling guidelines.

Migration Control:

IMPORTANT: For optimal use of the Normal Hb A2 Control with the CAPILLARYS 2 FLEX PIERCING system, use a tube specifically designed for blood controls and its corresponding cap (see EQUIPMENT AND

ACCESSORIES REQUIRED, Tubes and Caps for Controls) and identify this tube with a Normal Hb A2 Control bar code label.

Normal Hb A2 Use

The Normal Hb A2 Control should be used as follows:

- Add the reconstituted Normal Hb A2 Control into a tube designated as a blood control.
- Close the tube with its cap. Place a wedge adapter for the blood control tube in position #1 on the CAPILLARYS 2 FLEX PIERCING sample rack “zero” (No. 0) intended for control blood sample, containing a new green dilution segment.
- Place the tube with the Normal Hb A2 Control, identified with the Normal Hb A2 Control barcode label, on the wedge adapter on sample rack “zero” (No. 0).
- Start the analysis: Slide the sample rack “zero” (No. 0) into the CAPILLARYS 2 FLEX PIERCING system, select “Automatic Dilution” in the window which appears on the screen and validate.
- **Perform a second series of analyses with the control** a**fter changing the analysis buffer vial (even when the lot number is identical), after changing the technique, after a capillary cleaning sequence with CAPICLEAN, or after a software upgrade or after capillary activation** by immediately sliding the sample rack “zero” (No. 0)in again with the same dilution segment containing the Normal Hb A2 control, that was previously diluted during the first series and an empty tube for control identified with the Normal Hb A2 Control barcode label in position #1. In the window called “Hb A2 Normal Control”, appearing on the screen, select “Manual Dilution” and validate.

The results are then automatically recognized by the software for data analysis.

NOTE: For the first-time use of the HEMOGLOBIN(E) analysis program with the CAPILLARYS 2 FLEX PIERCING instrument, or after a prolonged stoppage (over one week), it is recommended to perform three (3) successive series of analyses with the Normal Hb A2 Control.

IMPORTANT: The Hb A fraction of the Normal Hb A2 Control must show a minimal optical density (OD) of 0.10 If the OD is less than 0.10, re-centering of the electrophoretic pattern will not occur correctly. If less than 0.10, identification of Hb fractions: Hb A, Hb F, Hb A2 and Hb C, and determination of the migration zone of other variants may not be possible or will be incorrect. (see RESULT ANALYSIS).

After the installation of the CAPILLARYS 2 FLEX PIERCING instrument, during the first sequence of blood sample analysis, a red warning signal will appear if Hb A is absent in a sample and the re-centering of the electrophoretic pattern will not be possible (see RESULT ANALYSIS). It is then recommended to analyze a blood sample with Hb A on the capillary involved, and to analyze the sample without Hb A again by placing it in a capillary which has already detected Hb A.

IMPORTANT: For optimal use of the Normal Hb A2 Control, use a barcode label intended to identify the tube used as the Hb A2 Control, close the tube with its specific cap before using it, and place it on the wedge adapter to the sample rack.

Utilization of a wedge adapter for conical tubes intended for controls:

This (yellow) wedge adapter is intended to support the conical tubes for blood controls on sample rack “zero” (No. 0) or on a rack of samples for the CAPILLARYS 2 FLEX PIERCING system. It has two markers which allow estimating of the volume of blood control available to perform the analysis:

1. When the tube is supported by the wedge adapter, the upper marker is located at the top of the wedge adapter and corresponds to a

volume of about 250µL blood control in the tube. When the volume of blood control reaches this level or higher, it is of sufficient volume to perform a complete analysis of this blood control with sample rack "zero" (No. 0).

2. When the tube is supported by the wedge adapter, the lower level is located at the bottom of the crenulation (tiny notch) and corresponds to a volume of about 100µL blood control in the tube. When the volume of the blood control reaches this level or lies between the two markers of the wedge adapter, it is of sufficient volume to run one blood control analysis on a sample rack.

v. Frequency of Running the Normal Hb A2 Control*

The Normal Hb A2 Control acts as the *migration control* before starting a new analysis sequence, after the analyses of ten (10) successive sample racks, and at the end of an analysis sequence. **The Normal Hb A2 control is performed with each batch of patient samples on each individual capillary chamber.The values obtained must fall within the assayed lot specific range provided by the manufacturer**. The Normal Hb A2 control results are reviewed and results must be considered acceptable with satisfactory separations (including acceptable optical density readings) prior to reporting patient results.

***** Refer to Normal Hb A2 Control Package Insert for additional details on using the Normal Hb A2 Control.

2. **The Pathological Hb A2 Control** – is used for the qualitative quality control for human Hb A2. It should be treated (with its bar code label for the CAPILLARYS procedures) like a normal human blood.

i. QC specimens are tested in the same manner as patient specimens and by the same personnel performing patient testing.

ii. QC should be handed and stored according to manufacturers' guidelines.
iii. Refer to the package insert details on reconstitution procedure, storage and handling of this control (IFU)

3. **The Hb AFSC Control** – is used:

-for quantitative quality control of the electrophoretic separation of human hemoglobin A, F, S , and C with the CAPILLARYS HEMOGLOBIN(E) used with CAPILLARYS 2 Flex-Piercing instruments

-for the qualitative quality control of electrophoretic separation of human hemoglobins A, F, S, and C with the CAPILLARYS HEMOGLOBIN(E) used with Sebia CAPILLARYS 2 Flex-Piercing instruments.

-It contains stabilizers and preservatives (including Chloramphenicol at a concentration no greater than 0.1 %) to maintain the stability of the hemoglobin fractions.

- The Hb AFSC Control should be used with its bar code label for the CAPILLARYS like normal human blood.

i. QC specimens are tested in the same manner as patient specimens and by the same personnel performing patient testing.
ii. QC should be handed and stored according to manufacturers' guidelines.
iii. Refer to the package insert details on reconstitution procedure, storage and handling of this control (IFU)

vi. Frequency of Running the Hb AFSC Control

1. The Hb AFSC control is performed with each batch of patient samples and on each individual capillary chamber. The values obtained must fall within the assayed lot specific range provided by the manufacturer.

QC Acceptable Range Verification for new lots of controls

i. The Normal Hb A2 Control, and the Hb AFSC Control are assayed controls with set limits defined by the manufacturer.

ii. Each lot of control must be verified (prior to quality control use) against the specified set ranges supplied by the manufacturer.

iii. New lots of controls are verified using the below guideline;

1. Manufacturer's guidelines should be followed for processing and handling control samples.
2. New lots of controls should be tested as patient samples (over each capillary chamber) to obtain numeric results for each analyte.
3. New lots of controls are considered valid for QC use, if the results observed are within the acceptable ranges defined by the manufacturer.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

1. The values obtained for controls must fall within the assayed lot specific range provided by the manufacturer.
2. Controls are reviewed and results must be considered acceptable with satisfactory separations (including acceptable optical density readings) prior to reporting patient results.
3. If results are outside of the acceptable limits or electrophoretic separation is unsatisfactory, **patient results should NOT be reported**. Patient samples are to re-analyzed once corrective actions has been taken and QC results are acceptable.
4. Corrective actions could include repeating QC with a new set of QC aliquots, or reconstitution a new vial of QC (of the same lot), or changing out the appropriate wash solutions, hemolysate wash, and/or buffers.
5. QC results must be acceptable prior to releasing patient results. If QC results are still unacceptable after performing corrective actions immediately, call the Sebia technical line at 1-800-835-6497 for additional technical trouble-shooting advice and alert the supervisor or delegate immediately.

6. All QC corrective actions taken should be documented.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- Only analyze blood samples contained in collection tubes under paragraph "EQUIPMENT AND ACCESSORIES REQUIRED" Call SEBIA technical service for further information on these devices.
- Avoid aged, improperly stored blood samples; degradation products, or artifacts may affect the electrophoretic pattern after seven (7) days storage.

IMPORTANT: Review the hematologic status of the patient in addition to evaluating results.

- The migration of a hemoglobin variant close to Hb A involves an under-estimation of the Hb A fraction, and that for the variant. Consequently, there is an overestimation of the A2 fraction. In order to accurately determine the Hb fraction, delete the separate integration of both variants and Hb A, and quantify these fractions together.
- Some homozygous-S subjects receive a "Hydrea"® (hydroxyurea) treatment that can induce synthesis of fetal hemoglobin. With the CAPILLARYS HEMOGLOBIN(E) procedure the mobility of the induced hemoglobin F is not different from that of physiological hemoglobin F.
- Due to the resolution and sensitivity limits of zone electrophoresis, it is possible that some hemoglobin variants may not be detected with this method.

The common interfering factors with the CAPILLARYS HEMOGLOBIN(E) procedure performed on the CAPILLARYS 2 FLEX-PIERCING instrument (triglycerides and bilirubin) were evaluated in studies based on the Clinical Laboratory Standards Institute (CLSI-USA) EP7-A2 guideline "Interference Testing in Clinical Chemistry".

The results are summarized below:

- No qualitative or quantitative interference was observed with the CAPILLARYS HEMOGLOBIN(E) procedure as performed on the CAPILLARYS 2 FLEX-PIERCING instrument for bilirubin concentrations equal to or less than 17.9 mg/mL, or 306 µmol/L.
- No qualitative or quantitative interference was observed with the CAPILLARYS HEMOGLOBIN(E) procedure performed on the CAPILLARYS 2 FLEX-PIERCING instrument for triglyceride concentrations equal to or less than 22.34 g/L.

13. REFERENCE RANGES (NORMAL VALUES)

Direct detection at 415 nm in the capillaries yields the relative percent (%) concentration of the individual hemoglobin zones. Reference values for individual major electrophoretic hemoglobin zones found in a healthy population of 113 adults (men and women) with normal values using an HPLC technique:

Hemoglobin A: 96.7 – 97.8%
Hemoglobin F: 0 – 1.4%
Hemoglobin A2: 2.2 – 3.2%
(*) See Interference and Limitations

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable for this procedure

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens should be store at 2-8°C for up to 7 days. Samples should not be stored at room temperature.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Not applicable for this procedure

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable for this procedure

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

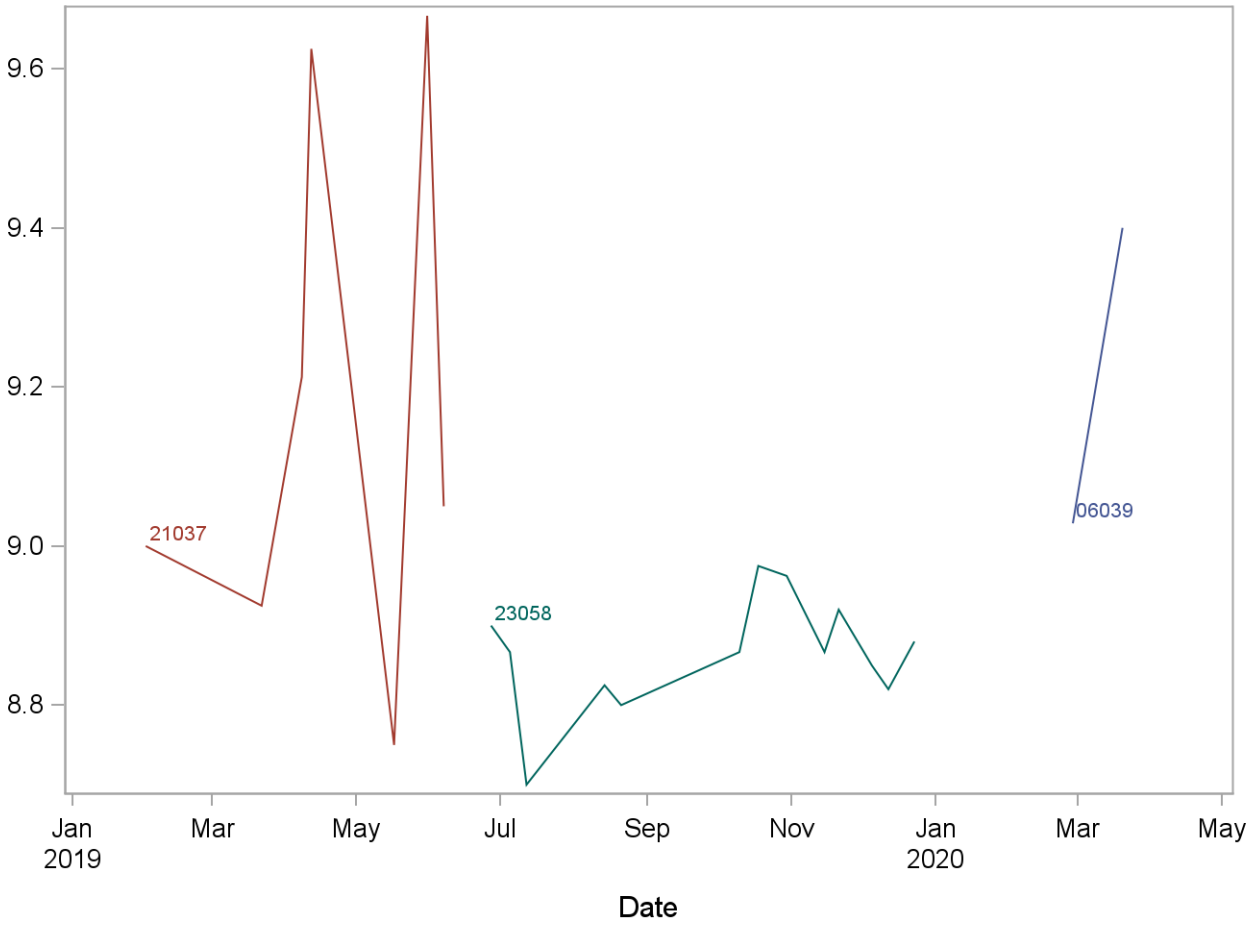
Not applicable for this procedure

19. SUMMARY STATISTICS AND QC GRAPHS

Please see following pages

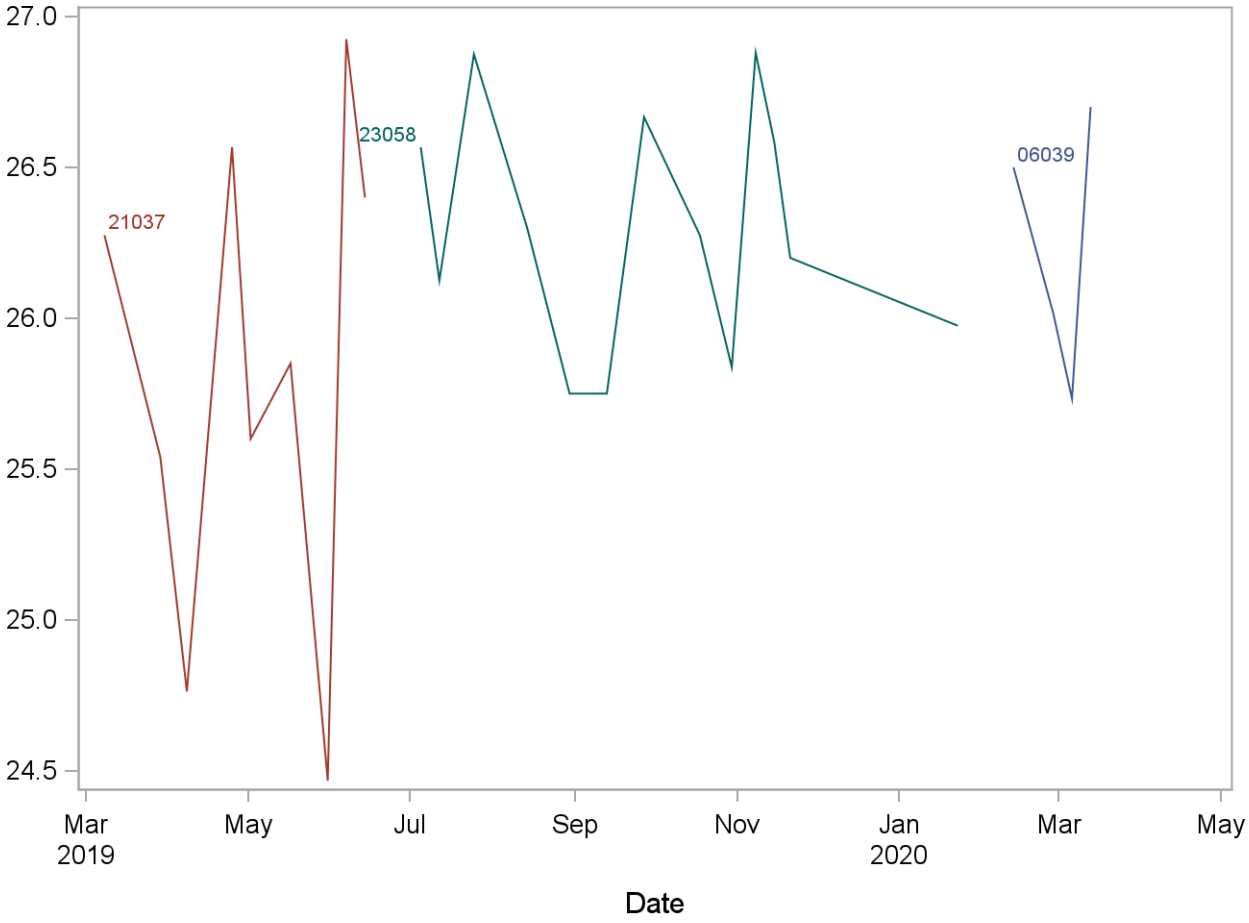
2019-2020 Summary Statistics and QC Chart
LBXVC (Hemoglobin Variant C (%))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
06039	2	28FEB20	20MAR20	9.21	0.26	2.9
21037	7	01FEB19	07JUN19	9.18	0.35	3.8
23058	13	27JUN19	23DEC19	8.86	0.07	0.8



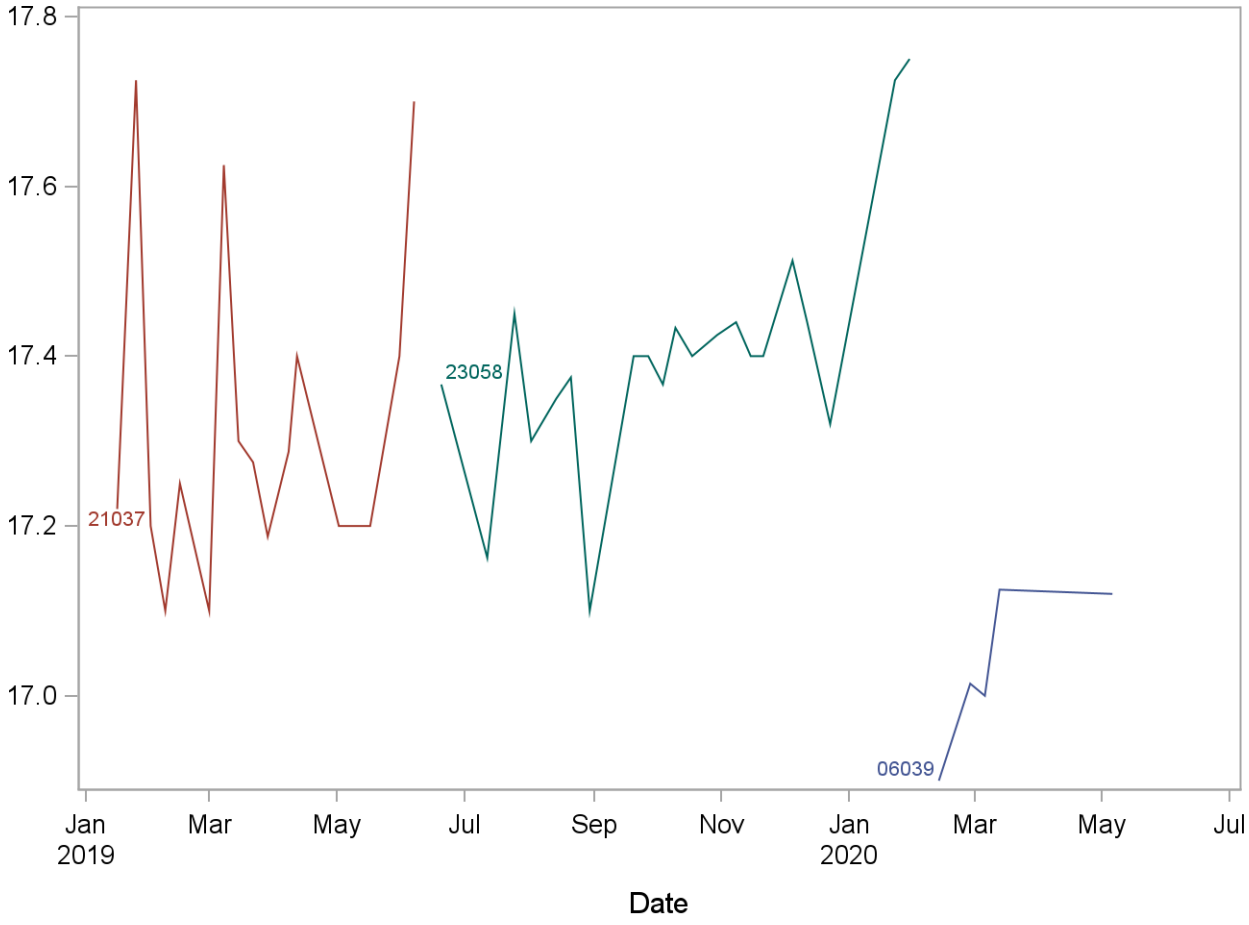
2019-2020 Summary Statistics and QC Chart
LBXVF (DHemoglobin Variant F (%))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
06039	4	13FEB20	13MAR20	26.24	0.44	1.7
21037	9	08MAR19	14JUN19	25.82	0.82	3.2
23058	13	05JUL19	23JAN20	26.29	0.40	1.5



2019-2020 Summary Statistics and QC Chart
LBXVS (Hemoglobin Variant S (%))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
06039	5	13FEB20	06MAY20	17.03	0.09	0.6
21037	16	16JAN19	07JUN19	17.32	0.20	1.1
23058	21	20JUN19	30JAN20	17.41	0.14	0.8



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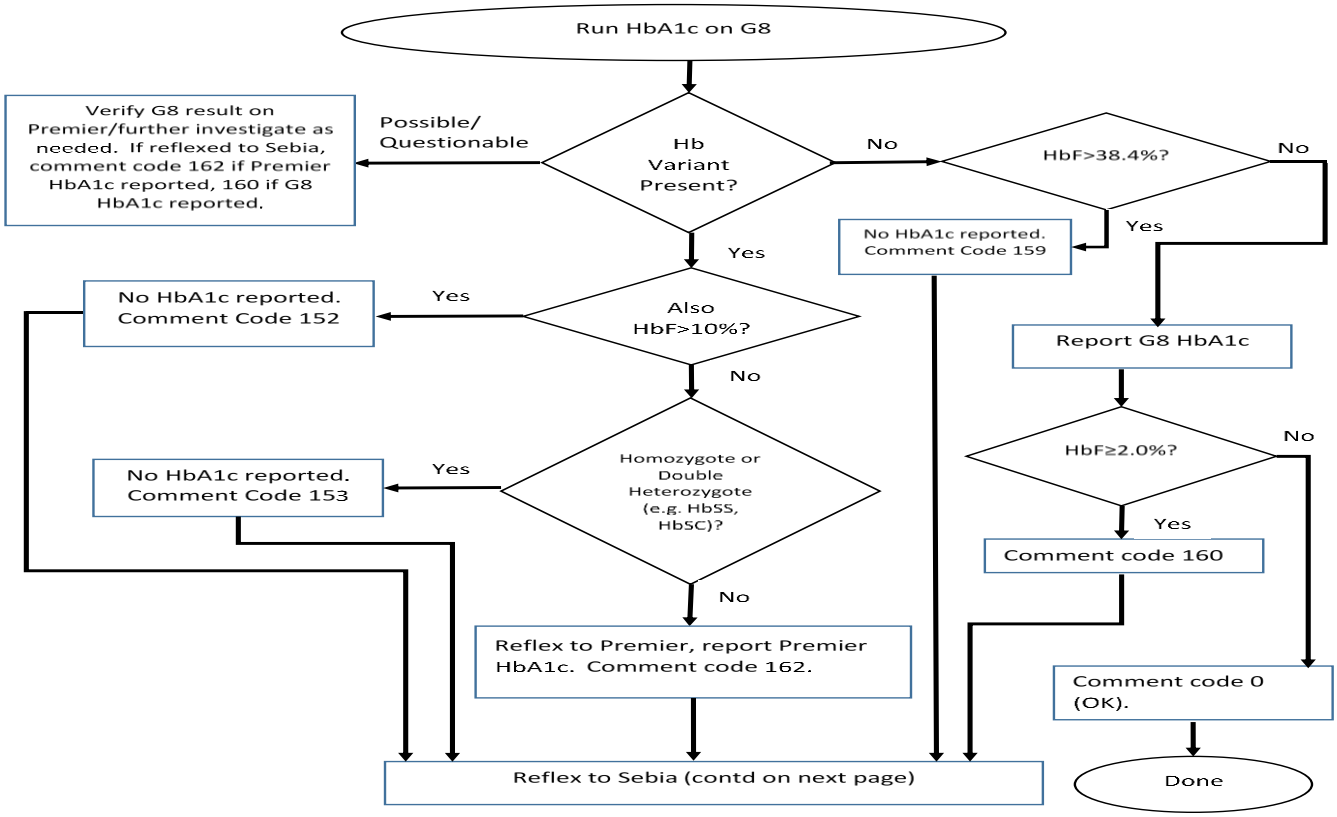
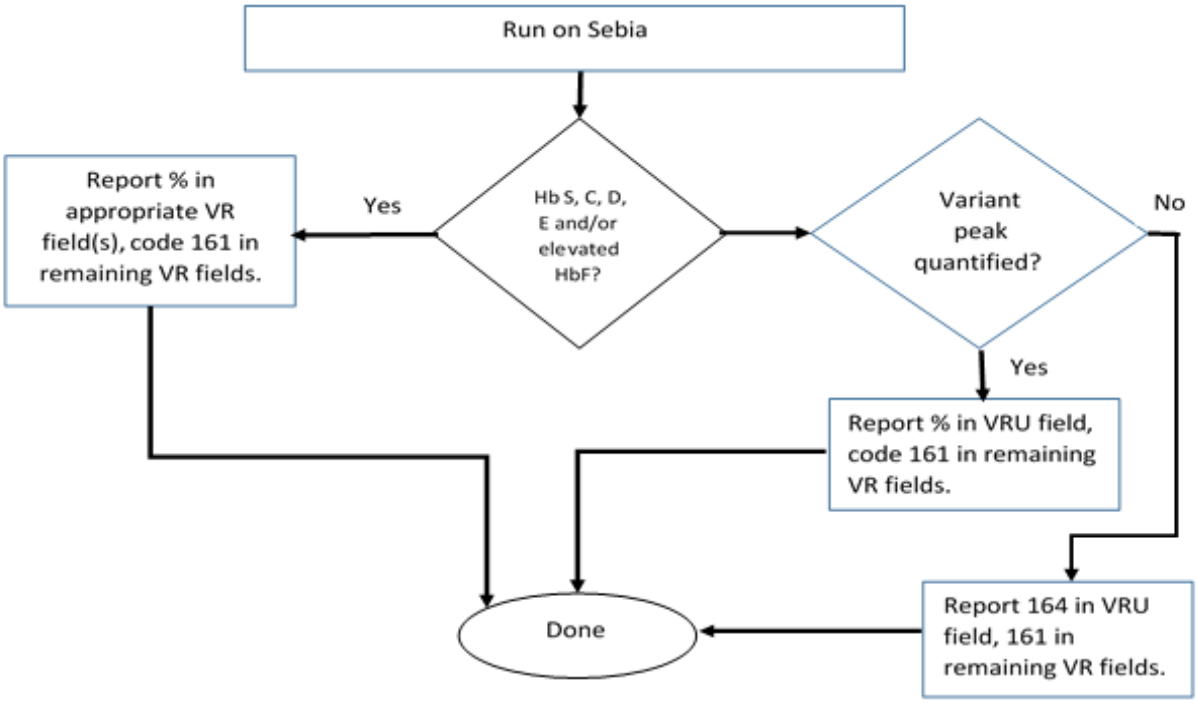
APPENDIX



HbA1c data files

152	Due the presence of both a variant hemoglobin and elevated HbF levels, we are unable to obtain a result free of analytical interference for this patient.
153	HBSS or HBSC or double Variant, not reportable
159	Hemoglobin variant or HbF levels present above instrument validation, result not reportable
160	HbF greater than or equal to 2.0%; G8 HbA1c reported
162	Abnormal variant detected – analyzed by boronate affinity method (reflexed testing method)



Variant data files

161	Variant Not Detected
164	Variant not quantified