CAPILLARYS HEMOGLOBIN(E)
Ref. 2007
INTENDED USE

The CAPILLARYS HEMOGLOBIN(E) kit is designed for the separation of the normal hemoglobins (A, F and A2) and for the detection of the major hemoglobin variants (especially S, C, E or D), by electrophoresis in alkaline buffer (pH 9.4) with the CAPILLARYS System. The CAPILLARYS performs all sequences automatically to obtain a complete hemoglobin profile for qualitative or quantitative analysis of hemoglobins. The assay is performed on sedimented, centrifuged or washed red blood cells; washing red blood cells is not essential to perform the analysis.

For In Vitro Diagnostic Use.

PRINCIPLE OF THE TEST

Hemoglobin is a complex molecule composed of two pairs of polypeptide chains. Each chain is linked to the heme, a tetapyrrolic 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: α2β2
- hemoglobin A2: α2δ2
- fetal hemoglobin F: α2γ2

The α-chain is common to these three hemoglobins.

The hemoglobin spatial structure and other molecular properties (like 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 charge 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. The CAPILLARYS 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.

The CAPILLARYS 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 electroosmotic flow. The CAPILLARYS System has capillaries functioning in parallel allowing 7 simultaneous analyses for hemoglobin quantification. A sample dilution with hemolysing solution is prepared and injected by aspiration at the anodic end of the capillary. A high voltage protein separation is then performed and direct detection of the hemoglobins is made at 415 nm at the cathodic end of the capillary. Before each run, the capillaries are washed with a Wash Solution and prepared for the next analysis with buffer.

The hemoglobins, separated in silica capillaries, are directly and specifically detected at an absorbance wave length of 415 nm which is specific to hemoglobins. The resulting electrophoregrams are evaluated visually for pattern abnormalities. Direct detection provides accurate relative quantification of individual hemoglobin fraction, with particular interest, such as A2 hemoglobin for β thalassemia diagnostic. In addition, the high resolution of this procedure should allow the identification of hemoglobin variants, in particular, to differentiate hemoglobins S from D, and E from C.

The hemoglobin A2 quantification can also be performed when hemoglobin E is present. By using alkaline pH buffer, normal and abnormal (or variant) hemoglobins are detected in the following order, from cathode to anode: δA2 (A2 variant), C, A2/O-Arab, E, S, D, G-Philadelphia, F, A, Hope, Bart, J, N-Baltimore and H.

The carbonic anhydrase is not visualized on the hemoglobin electrophoretic patterns, this permits to identify hemoglobin A2 variants in this migration zone.

REAGENTS AND MATERIALS SUPPLIED IN THE CAPILLARYS HEMOGLOBIN(E) KIT

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN. 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (ready to use)</td>
<td>2 vials, 700 mL each</td>
</tr>
<tr>
<td>Hemolyzing solution (ready to use)</td>
<td>1 vial, 440 mL</td>
</tr>
<tr>
<td>Wash solution (stock solution)</td>
<td>1 vial, 70 mL</td>
</tr>
<tr>
<td>Dilution segments</td>
<td>1 pack of 90</td>
</tr>
<tr>
<td>Filters</td>
<td>3 filters</td>
</tr>
</tbody>
</table>

630 tests based on maximum usage.

FOR OPTIMAL RESULTS:

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

PLEASE READ THE PACKAGE INSERT CAREFULLY.

1. BUFFER

Preparation

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

Use

Buffer for analysis of hemoglobins in CAPILLARYS.
Storage, stability and signs of deterioration
Store the buffer refrigerated (2 to 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.
NOTE: When stored at 2 – 8 °C, it is recommended to allow the buffer to come to room temperature prior to use.
Once the buffer vial has been opened and positioned on the CAPILLARYS system, it is stable for a maximum of 1 month (accumulated) at room temperature (15 to 30 °C). After each use, the buffer must imperatively be stored refrigerated (between 2 and 8 °C) without any delay, it is then stable until the expiration date indicated on the buffer vial label.
IMPORTANT: The accumulated time of the buffer stored at room temperature must not exceed 1 month.
Discard buffer if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

2. HEMOLYSING SOLUTION
Preparation
Hemolysing Solution is ready to use. It is a buffer with additives, nonhazardous at the concentration used, necessary for optimum performance.
Use
To dilute and hemolyze red blood cells.
Storage, stability and signs of deterioration
Store Hemolyzing Solution refrigerated (2 to 8 °C). It is stable until the expiration date indicated on the kit package or Hemolyzing Solution vial label. Avoid storage at room temperature or close to a window or to a heat source. DO NOT FREEZE.
Discard Hemolyzing Solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.
IMPORTANT: After each use, close immediately and tightly the hemolyzing solution vial and store it refrigerated.
NOTE: The usual color of hemolyzing solution is yellow.

3. WASH SOLUTION
Preparation
The vial of the stock wash solution should be diluted up to 700 mL with distilled or deionized water.
Use
For washing the capillaries before and after hemoglobin electrophoresis.
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 3 months.
Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

4. DILUTION SEGMENTS
Use
Coloured single use segments for blood sample dilution on the automated instrument. They are specific for CAPILLARYS HEMOGLOBIN(E) procedure.
WARNING: Dilution segments with biological samples have to be handled with care.

5. FILTERS
Use
Disposable filters for filtration of analysis buffer, working wash solution and distilled water (used for capillaries rinsing).
IMPORTANT: With each new kit, always change all the three filters.
Screw one filter at the connector situated at the extremity of each tube that plunges in the vials of buffer, wash solution and distilled or deionized water. When setting filters on CAPILLARYS system, rinse the connectors and the tubes with distilled or deionized water. Used filters must be rinsed before discard.
The filter intended for analysis buffer must be used for filtration of both buffer vials; the two other filters are intended for filtration of working wash solution and for distilled or deionized water (for capillary rinsing).
Storage
Before use, store the filters in their sealed package in a dry place at room temperature or refrigerated.

REAGENTS REQUIRED BUT NOT SUPPLIED
1. NORMAL Hb A2 CONTROL
Composition
The Normal Hb A2 Control (SEBIA, PN 4778) is obtained from a pool of normal human blood samples. The Normal Hb A2 Control is in a stabilized lyophilised form.
Intended use
The Normal Hb A2 Control is designed for the migration control before starting a new analysis sequence, and for the quality control of human hemoglobin A2 quantification with CAPILLARYS HEMOGLOBIN(E) electrophoresis procedure. Reconstitute each Normal Hb A2 Control vial with the exact volume of distilled or deionized water, as indicated in the package insert of the Normal Hb A2 Control. Allow to stand for 30 minutes and mix gently (avoid formation of foam).
Migration control: The Normal Hb A2 Control should be used as follows:
- Apply the reconstituted Normal Hb A2 Control in a microtube.
- Cut the cap of the microtube.
- Place the microtube, located on a new hemolyzing tube used as a holder, in position No. 1 on the CAPILLARYS sample rack No. 0 intended for control blood sample, and a new green dilution segment.
- Pour 4 mL CAPILLARYS HEMOGLOBIN(E) hemolyzing solution in a hemolyzing tube without introducing air bubbles and place it in position No. 8 on the sample rack No. 0.

IMPORTANT: Ensure the absence of foam in the tube before placing it on the sample rack.
- Start the analysis: Slide the sample rack No. 0 into the CAPILLARYS system, select “Automatic dilution” in the window which appears on the screen and validate.
- After having changed the analysis buffer vial (even if the lot number is unchanged) or the technique, after a capillary cleaning sequence with CAPICLEAN or after capillaries activation, perform a second series of analyses with the control, by sliding in again immediately the sample rack No. 0 with the same dilution segment containing the Normal Hb A2 Control, previously diluted during the first series, select in the window which appears on the screen “Hb A2 Normal Control”, “Manual dilution” and validate. The results are then automatically considered by the software for the data analysis.

IMPORTANT: For optimal use of the Normal Hb A2 Control, it is necessary to use one bar code label intended to identify the hemolyzing tube holding the microtube which contains the Hb A2 Control (cut the cap of the microtube before using it).

NOTES: For the first use of the “HEMOGLOBIN(E)” analysis program with the CAPILLARYS instrument, it is recommended to perform 3 successive series of analyses with the Normal Hb A2 Control.
After the installation of CAPILLARYS instrument, during the first sequence of blood sample analysis, a red warning signal will appear if hemoglobin A is absent in one sample (and the recentering of the electrophoretic pattern will not be possible, see paragraph “Result analysis”).
It is then recommended to analyze a blood sample with hemoglobin A on the concerned capillary and to analyze again the sample without hemoglobin A by placing it in a position corresponding to a capillary which has already detected hemoglobin A.

Quality control: The Normal Hb A2 Control should be used as a normal human blood. After reconstitution, use directly the Normal Hb A2 Control as a blood sample to analyze or as a migration control (with the sample rack No. 0, see paragraph before). It will be automatically diluted with hemolyzing solution. It is recommended to include one analysis of Normal Hb A2 Control. The values obtained must fall within the range provided with each batch of Hb A2 Control.

IMPORTANT: For optimal use of the Normal Hb A2 Control placed on a sample rack, it is necessary to use one bar code label intended to identify the hemolyzing tube holding the microtube which contains the Hb A2 Control (cut the cap of the microtube before using it).

Storage, stability and signs of deterioration
Before reconstitution, store the lyophilised Normal Hb A2 Control refrigerated (2 to 8 °C). It is stable until the expiration date indicated on the box or vial labels.
Store the reconstituted Normal Hb A2 Control at 2 – 8 °C. Due to the risk of microbial contamination and denaturation, use it within one week.
The reconstituted Control may also be frozen (in aliquots) and stored at -20 °C for 6 months maximum.

IMPORTANT: After storage at 2 – 8 °C or at -20 °C, homogenize the reconstituted Normal Hb A2 Control before the analysis with the CAPILLARYS system.

NOTE: For optimal use with the CAPILLARYS system, it is recommended to split the Control into aliquots in microtubes before freezing.
Before use, store the thawed Normal Hb A2 Control at 2 – 8 °C and use it within the day. Do not freeze and thaw the Control more than 15 times.
The hemolyzed Normal Hb A2 Control should be stored at 2 – 8 °C and used within one day.
The dilution segment containing the hemolyzed Normal Hb A2 Control may be frozen and stored at –20 °C. Do not freeze and thaw the dilution segment with hemolyzed Control more than twice.

IMPORTANT: Do not leave the dilution segment with hemolyzed Control at room temperature.

NOTE: During transportation, the lyophilized Normal Hb A2 Control can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

No test method can provide an absolute assurance of the absence of HIV, hepatitis B and C or other infectious agents. Therefore, handle the Normal Hb A2 Control as a hazardous biological material.
This lot of Control was found negative:
- against hepatitis B surface antigen;
- for antibody to HCV;
- for antibody to HIV1 and HIV2.

2. DISTILLED OR DEIONIZED WATER
Use
For rinsing capillaries in automated system CAPILLARYS, SEBIA, for capillary electrophoresis. It is recommended to filter distilled or deionized water with 0.45 µm filter before use.
To prevent microbial proliferation, change the water every day. In case of longer storage, add 3.5 µL/dL of ProClin 300.

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

3. CAPICLEAN
Composition
The vial of CAPICLEAN concentrated solution (SEBIA, PN 2051, 12 mL) contains: proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, necessary for optimum performances.

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

Use
For weekly capillaries and sample probe cleaning in automated system CAPILLARYS, SEBIA, for capillary electrophoresis. When using “100 µL” dilution segments specific for CAPILLARYS HEMOGLOBIN(E) procedure, apply 50 µL of concentrated CAPICLEAN solution and 50 µL of distilled or deionized water stored at room temperature, to each well, avoid formation of foam and follow the procedure.

See the instruction sheets of CAPICLEAN, SEBIA.
IMPORTANT: Do not re-use the dilution segment after capillaries and 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.
CAPICLEAN must be free of precipitate. Discard CAPICLEAN if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

4. SODIUM HYPOCHLORITE SOLUTION (for sample probe cleaning)
Preparation
Prepare a 9° chlorinated sodium hypochlorite solution (2 % to 3 % chloride) by diluting 250 mL 36° chlorinated concentrated solution (9.6 % chloride) to 1 liter with cold distilled or deionized water.
Use
For the sample probe cleaning in the CAPILLARYS System (weekly maintenance in order to eliminate adsorbed proteins from the probe).
See the instruction sheets of CAPILLARYS, SEBIA.
• Use the sample rack designed for the maintenance (No. 100).
• Place a tube containing 2 mL diluted chlorinated solution previously prepared, in position No. 1 on this sample rack.
• Slide the sample rack No. 100 for maintenance in the CAPILLARYS System.
• In the "MAINTENANCE" window which appears on the screen, select "Launch the probe cleaning (chlorinated sodium hypochlorite solution or CDT wash 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 1 year. Avoid storage in sunlight, close to heat and ignition source, and to acids and ammonia.

5. CAPILLARYS WASH SOLUTION
Preparation
Each vial of the stock CAPILLARYS Wash Solution (SEBIA, PN 2052, 2 vials, 70 mL) should be diluted up to 700 mL with distilled or deionized water.
Use
For washing the capillaries of CAPILLARYS. This additional reagent is needed when the number of tests in series is below 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 3 months. Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

6. SALINE
Preparation
Make 0.15 M (0.9 g/dL) NaCl solution in distilled or deionized water.
Use
To wash red blood cells before storage at - 80 °C, if necessary.

Storage, stability and signs of deterioration
Store saline at room temperature or refrigerated. Discard after 3 months or if it changes its appearance, e.g., becomes cloudy due to microbial contamination. For longer storage periods, add sodium azide, 0.1 g/dL.

EQUIPMENT AND ACCESSORIES REQUIRED

1. CAPILLARYS System SEBIA, PN 1220 or PN 1222.
2. Sample racks supplied with CAPILLARYS.
3. Container Kit supplied with CAPILLARYS: Rinse (to fill with distilled or deionized water), wash solution and waste container.

SAMPLES FOR ANALYSIS

Sample collection and storage
Fresh anticoagulated blood samples are recommended for analysis. Common anticoagulants such as those containing EDTA, citrate or heparin are acceptable; avoid those with iodoacetate. Blood must be collected according to established procedures used in clinical laboratory testing. Samples may be stored for up to 7 days between 2 and 8 °C.
For longer storage, samples can be frozen at - 80 °C within 8 hours of collection after having washed the red blood cells according to the following procedure: Centrifuge anticoagulated blood at 5 000 rpm for 5 minutes; discard the plasma; wash the red blood cells (RBC) 2 times with 10 volumes of saline (centrifuge after each washing step); discard the excess of saline over the red blood cells pellet and vortex them before freezing.
Frozen blood samples are stable for 3 months maximum at - 80 °C.
IMPORTANT: For optimal storage of blood samples, store them at -80 °C. Do not store at -20 °C (see BIBLIOGRAPHY, J. Bardakdjian-Michau et al, 2003).
NOTE: Samples should not be stored at room temperature!
Progressive hemoglobins (Hb) degradation may occur for samples stored between 2 to 8 °C.
When the blood sample is stored for more than 7 days at 2 - 8 °C:
• a weak fraction, corresponding to methemoglobin, appears in the Hb S migration zone,
• when Hb C is present, a fraction corresponding to degraded Hb C appears more anodic than Hb A2 which does not interfere with it (Z4 zone, see the table in paragraph "Interpretation"),
• when Hb O-Arab is present, a fraction corresponding to degraded Hb O-Arab appears in the Hb S migration zone (Z5 zone, see the table in paragraph “Interpretation”),
• when Hb E is present, a fraction corresponding to degraded Hb E appears in the Z6 zone (see the table in paragraph “Interpretation”),
• when Hb S is present, a fraction corresponding to degraded Hb S appears in the Hb F migration zone (Z7 zone, see the table in paragraph “Interpretation”),
• 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”).

When Hb F is present (in blood samples from newborn babies), a fraction appears in the Hb A migration zone (Z9 zone, see the table in paragraph “Interpretation”) due to the sample degradation.

When stored for more than 10 days, viscous aggregates in red blood cells are observed; it is necessary to discard them before the analysis.

Sample preparation
• Let red blood cells precipitate for several hours at 2 - 8 °C or centrifuge the blood sample at 5 000 rpm for 5 minutes.
• Discard carefully the maximum volume of plasma (for samples collected with heparin, discard viscous aggregates located between plasma and red blood cells).
• Vortex for 5 seconds.

IMPORTANT: Do not use blood samples containing 3 mm maximum residual plasma over red blood cells; when more than 3 mm plasma is present in the tube, the analysis should be affected.

Particular cases: Analysis of samples without any Hb A or Hb A2 (these samples are perfectly quantified but not identified by zones).
To identify hemoglobin fractions of a sample without any hemoglobin A or hemoglobin A2, it is recommended to prepare this sample according to one of the two following procedures:
Automatic dilution:
- In a microtube, mix one volume (80 μL) of red blood cells from the sample to analyze with one volume of Normal Hb A2 Control (80 μL).
- Vortex for 5 seconds.
- Cut the cap of the microtube.
- Place the microtube, on a new hemolysing tube used as a support-tube, on a sample rack of the CAPILLARYS system.
- Perform the analysis of this sample according to the standard procedure like a usual blood sample.

Manual dilution:
- Apply, directly in the wells of a new green dilution segment, 9 μL of reconstituted Normal Hb A2 Control with 9 μL of blood sample to analyze and 90 μL of CAPILLARYS HEMOGLOBIN(E) hemolyzing solution.
- Mix by repeated pipettings.
- Place this dilution segment on the sample rack No. 0 of CAPILLARYS.
- Slide the sample rack into the CAPILLARYS system, select “Sample” with “manual dilution” in the window which appears on the screen and validate.

IMPORTANT: Do not use unsedimented blood samples.
• Avoid aged, improperly stored blood samples; the automated hemolysis of samples may be disturbed by viscous aggregates in red blood cells.
Then, degradation products (as artefacts) may affect the electrophoretic pattern.

PROCEDURE
The CAPILLARYS system is a multiparameter instrument for hemoglobins analysis on parallel capillaries. The hemoglobins assay uses 7 of the total 8 instrument capillaries to run the samples.
The sequence of automated steps is as follows:
• Bar code reading of sample tubes (for up to 7 tubes) and samples-racks;
• Sample hemolysis and dilution from primary tubes (without any plasma) into dilution segments;
• Capillary washing;
• Injection of hemolized samples;
• Hemoglobin separation and direct detection of the separated hemoglobins on capillaries.

The manual steps include:
• Placement of opened sample tubes in sample-racks in positions 1 to 7;
• Placement of hemolysing solution tube in sample-racks in position 8;
• Placement of new dilution segments in sample-racks;
• Placement of racks on the CAPILLARYS instrument;
• Removal of sample-racks after analysis.

PLEASE CAREFULLY READ THE CAPILLARYS INSTRUCTION MANUAL.

I. PREPARATION OF CAPILLARYS ANALYSIS
1. Switch on CAPILLARYS instrument and computer.
2. Set up the software, enter and the instrument automatically starts.
3. The CAPILLARYS HEMOGLOBIN(E) kit is intended to run with “HEMOGLOBIN(E)” analysis program from the CAPILLARYS instrument. To select “HEMOGLOBIN(E)” analysis program and place the CAPILLARYS HEMOGLOBIN(E) buffer vial in the instrument, please read carefully the CAPILLARYS instruction manual.
4. The sample rack contains eight positions for sample tubes. Place seven opened sample tubes without any plasma on each sample rack; the bar code of each tube must be visible in the openings of the sample rack.

IMPORTANT: If the number of tubes to analyze is less than 7, complete the sample rack with tubes containing distilled or deionized water.
5. Pour 4 mL CAPILLARYS HEMOGLOBIN(E) hemolysing solution in a tube without introducing air bubbles and place it in position No. 8 on the sample rack.

**IMPORTANT:** Ensure the absence of foam in the tube before placing it on the sample rack.

6. Position a new dilution segment on each sample rack. A message will be displayed if the segment is missing.

7. Slide the complete sample carrier(s) into the CAPILLARYS system through the opening in the middle of the instrument. Up to 13 sample racks can be introduced successively and continuously into the system. It is advised to use the sample rack No. 0 intended for control blood sample.

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

9. Take off carefully used dilution segments from the sample rack and discard them.

**WARNING:** Dilution segments with biological samples have to be handled with care.

### DILUTION - MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

1. Bar codes are read on both sample tubes and sample racks.
2. Samples are diluted in hemolysing solution and the dilution needle is rinsed after each sample.
3. Capillaries are washed.
4. Diluted samples are injected into capillaries.
5. Migration is carried out under constant voltage for about 8 minutes and the temperature is controlled by Peltier effect.
6. Hemoglobins 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 introduced sample rack. The electrophoretic patterns appear after about 20 minutes from the start of the analysis. For the following sample rack, the first two steps (bar code reading and sample dilution) are performed during analysis of the previous sample rack.

### II. RESULT ANALYSIS

At the end of the analysis, relative quantification of individual hemoglobin fractions is performed automatically and profiles can be analyzed: the hemoglobin fractions, Hb A, Hb F and Hb A2 are automatically identified; the Hb A fraction is adjusted in the middle of the review window. The resulting electrophoregrams are evaluated visually for pattern abnormalities.

The potential positions of the different hemoglobin variants (identified in zones called Z1 to Z15) are shown on the screen of the system and indicated on the result ticket. The table in paragraph “Interpretation” shows known variants which may be present in each corresponding zone.

When the software identifies a hemoglobin fraction in a defined zone, the name of this zone is framed.

Patterns are automatically adjusted with regard to Hb A fraction to facilitate their interpretation:
- when Hb A and / or Hb A2 fractions are not detected on an electrophoretic pattern, a yellow warning signal appears, the adjustment is performed using the position of the Hb A fraction on the two previous patterns obtained with the same capillary; then, there is no fraction 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 any detection of Hb A, the yellow warning signal does not appear, the adjustment is then performed using the position of the Hb F fraction, and 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).

In those three cases, the different variant zones (Z1 to Z15) do not appear neither on the screen of the system, nor on the ticket result.

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 with the native curve. This display allows the Hb A2 fraction quantification if Hb C is present in the sample.

**WARNING:** In some cases of homozygous hemoglobin C, hemoglobin A2 is not fitted and then not accurately quantified if hemoglobin C is present in the sample.

PLEASE CAREFULLY READ THE CAPILLARYS INSTRUCTION MANUAL.

### III. END OF ANALYSIS SEQUENCE

At the end of each analysis sequence, the operator must initiate the “stand by” or “shut down” procedure of the CAPILLARYS system in order to store capillaries in optimal conditions.

### IV. FILLING OF REAGENT CONTAINERS

The CAPILLARYS system has a reagent automatic control.

**IMPORTANT:** Please refer to the instructions for replacement of reagent containers respecting color code 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 and / or ;
- Fill the container with working wash solution and / or ;
- Fill the container with filtered distilled or deionized water for rinsing capillaries and / or ;
- Empty the waste container.

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

PLEASE CAREFULLY READ THE CAPILLARYS INSTRUCTION MANUAL.

### QUALITY CONTROL

After having changed the analysis buffer lot number or the technique, or after a capillary cleaning sequence with CAPICLEAN, **and before starting a new analysis sequence**, it is necessary to run two analysis sequences with the Normal Hb A2 Control, SEBIA, PN 4478, and the sample rack No. 0 intended for control blood sample (see paragraph REAGENTS REQUIRED BUT NOT SUPPLIED).

It is also advised to include into each run of samples, an assayed control blood (for example, a blood sample containing hemoglobins A, F, C and S, such as Hb AFSC Control, SEBIA, PN 4792, or a normal blood sample, the Normal Hb A2 Control, SEBIA, PN 4778 or the Pathological Hb A2 Control, SEBIA, PN 4779).
RESULTS

Values
Direct detection at 415 nm in capillaries yields relative concentrations (percentages) of individual hemoglobin zones.
Normal values for individual major electrophoretic hemoglobin zones in the CAPILLARYS system have been established from a healthy population of 113 adults (men and women) with normal hemoglobin values using HPLC technique:

- Hemoglobin A: comprised between 96.8 and 97.8 %
- Hemoglobin F: < 0.5 % (*)
- Hemoglobin A2: comprised between 2.2 and 3.2 %

(*) See Interference and limitations.
It is recommended that each laboratory establish its own threshold values.

NOTE: Normal values have been established using the standard parameters of the CAPILLARYS software (smoothing 0 and hemoglobin fractions automatic quantification with HEMOGLOBIN(E) analysis program).

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

Interpretation
See ELECTROPHORETIC PATTERNS, figures 1 – 15.
The different migration zones of hemoglobin variants (called Z1 to Z15) are shown on the screen of the system and on the result ticket. Passing the mouse cursor over a zone name displays icon information containing possible hemoglobin variants that could be seen in this zone. For each fraction, the maximum position defines the migration zone.
The following table presents potential variants in each zone:

<table>
<thead>
<tr>
<th>Zone</th>
<th>Hemoglobins (Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z1</td>
<td>Hb S, Hb A2, Hb A2 variant, Winnipeg Hb A2 variant, Q-Thailand Hb A2 variant, other Hb A2 variants</td>
</tr>
<tr>
<td>Z2</td>
<td>Hb C, Hb Constant Spring, Setif Hb A2 variant</td>
</tr>
<tr>
<td>Z3</td>
<td>Hb A2, Hb O-Arab</td>
</tr>
<tr>
<td>Z4</td>
<td>Hb E, Hb Köln, Hb A2 variants, degraded Hb C</td>
</tr>
<tr>
<td>Z5</td>
<td>Hb S, Hb Hasharon, degraded Hb O-Arab</td>
</tr>
<tr>
<td>Z6</td>
<td>Hb D-Punjab (D-Los Angeles), Hb Osu Christiansborg, Hb D-Ouled Rabah, Hb Lepore, Hb G-Philadelphia, Hb Korne-Bu (G-Accra), Hb Köln, Hb G-Taipei, Hb Winnipeg, Hb Setif, J-Toronto Hb A2 variant, J-Rovigo Hb A2 variant, degraded Hb E</td>
</tr>
<tr>
<td>Z7</td>
<td>Hb F, Hb Q-Thailand (G-Taichung), Hb Richmond, Hb G-San José, Hb Porto-Alegre, Hb Presbyterian, degraded Hb S</td>
</tr>
<tr>
<td>Z8</td>
<td>Acetylated Hb F, Hb Atlanta</td>
</tr>
<tr>
<td>Z9</td>
<td>Hb A, Hb Phnom Penh, Hb Toulon, Hb Okayama, Hb Fontainebleau, Hb Raleigh, Hb Hekinan, Hb Camperdown</td>
</tr>
<tr>
<td>Z10</td>
<td>Hb Hope</td>
</tr>
<tr>
<td>Z11</td>
<td>Degraded Hb A, Hb J-Kaohsiung</td>
</tr>
<tr>
<td>Z12</td>
<td>Hb Bart's, Hb J-Providence, Hb J-Broussais, Hb J-Toronto, Hb J-Meinung (J-Bangkok), Hb J-Mexico, Hb J-Baltimore</td>
</tr>
<tr>
<td>Z13</td>
<td>Hb J-Rovigo, Hb N-Baltimore</td>
</tr>
<tr>
<td>Z15</td>
<td>Hb H</td>
</tr>
</tbody>
</table>

WARNING: The graduation of the horizontal axis does not allow, in any case, the identification of an 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, 12). The clinical significance of such a change depends on the type of amino acid and the site involved(13). In clinically significant disease, either the α-chain or the β-chain is affected.
More than 900 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 an easy detection by electrophoresis.
There are five main abnormal hemoglobins which present a 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. It is due to the replacement of one glutamic acid (an acidic amino acid No. 6) of the β-chain by valine (a neutral amino acid): when compared to Hb A, its isoelectric point is elevated and its total negative charge decreased with the analysis pH. Its electrophoretic mobility is therefore increased in the capillary and this hemoglobin is faster than A fraction.
With alkaline buffered CAPILLARYS HEMOGLOBIN(E) procedure, hemoglobin S migrates between A and A2 fractions, next to Hb A2.

Hemoglobin C
One glutamic acid of the β-chain is replaced by lysine (a basic amino acid No. 6): its mobility is strongly reduced. When compared to Hb A, its isoelectric point is highly elevated and its total negative charge decreased with the analysis pH. Its electrophoretic mobility is therefore increased in the capillary and this hemoglobin is faster than A fraction which allows its differentiation. Hemoglobins C; E and O-Arab are not superimposed on the electrophoretic pattern and are easily identified.

Hemoglobin E
One glutamic acid of the β-chain (No. 26) is replaced by lysine. With CAPILLARYS HEMOGLOBIN(E) procedure, hemoglobin E migrates just anodically behind hemoglobin A2 and is totally separated from it. Then, when hemoglobin E is present, A2 fraction can be measured to detect β thalassemia.
Hemoglobin O-Arab
One glutamic acid of the β-chain (No. 121) is replaced by lysine. With CAPILLARYS HEMOGLOBIN(E) procedure, hemoglobin O-Arab migrates exactly like hemoglobin A2. In such a case, hemoglobin A2 cannot be quantified. When this 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)
One glutamic acid of the β-chain (No. 121) is replaced by glutamine. With CAPILLARYS HEMOGLOBIN(E) procedure, hemoglobin D (called D-Punjab, D-Los Angeles, D-Chicago or D-Dubai) migrates behind hemoglobin S, this property allows to differentiate S and D hemoglobins.

2. Quantitative abnormalities: Thalassemias
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-thalassemias
They are characterized by the decrease of synthesis of the α-chains, consequently affecting the synthesis of all normal hemoglobins. The excess of synthesis of the β- and γ-chains in relation to α-chains induces the formation of tetrameres without any α-chain:
• hemoglobin Bart = γ 4,
• hemoglobin H = 8 A.
Hemoglobin H presents a low isoelectric point ; with CAPILLARYS HEMOGLOBIN(E) procedure, it migrates more anodic than hemoglobin A (and may appear as one or several fractions).

Beta-thalassemias
They are characterized by the decrease of synthesis of the β-chains. Only hemoglobin A synthesis is affected. Therefore hemoglobin F and hemoglobin A2 percentages are increased with respect to hemoglobin A. With CAPILLARYS HEMOGLOBIN(E) procedure, values obtained for different normal hemoglobin fractions allow the detection of beta thalassemias.

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 (which represents about 15 to 25 % of hemoglobin F). The CAPILLARYS system can identify this acetylated hemoglobin separately from the hemoglobin A without any confusion.
• When a small fraction (about 0.5 to 3 %) migrates between hemoglobins F and A2 (A2 variant), a hemoglobin A2 variant may be suspected.
• When a hemoglobin A2 variant is detected (αA2 or any other A2 variant), it is recommended to add its percentage to hemoglobin A2 for a better beta-thalassemia diagnostic.

• Some hemoglobin variants (such as Hb Camperdown and Hb Okayama) 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 which migrate in zone Z12 are sometimes quantified with imprecision (too asymmetric Hb Bart’s, for example). It is thus necessary to delete automatic quantification and then to quantify them manually.
• When analysing blood samples from newborn babies, Hb A from samples containing Hb F at high concentrations may be disturbed, especially due to the presence of degraded Hb F in its migration zone. The Hb A percentage indicated by the software may be overvalued. In addition, 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 levels (> 60 %), it is necessary to perform complementary analyses in order to confirm the presence of Hb A.
• For newborn babies until 6 – 9 months old, it is recommended to analyze many blood samples (collected monthly, for example) in order to check the Hb F concentration. It will allow to verify the decrease of Hb F concentration and the potential presence of a variant.

In case of uncertainty, it is advised to confirm by using complementary studies and to analyze parents' blood samples.

• Examples with increased hemoglobin F (Hb F) (except for newborn babies):
  - pregnancy;
  - patients with sickle cell disease, more than 2 years old, with a Hydrea® (hydroxyurea) treatment and / or transfused and / or producing naturally Hb F increased by compensation;
  - patients, aged more than 2 years old, with HPFH trouble (hereditary persistence of foetal hemoglobin exhibiting 20 to 40 % Hb F for heterozygous patients);
  - patients, more than 2 years old, with leukaemia (with any type), hereditary haemolytic anaemia, diabetes, thyroid disease, hyperactivity of bone marrow, multiple myeloma, cancer with metastases.

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

• When analysing blood samples from transfused patients with sickle cell disease, with low Hb A level (< 10 %), Hb S fraction may appear shifted from Z5 zone to Z8 zone. It is necessary to analyze the hematologic state and to perform complementary studies in order to confirm the presence of Hb S.

Interference and Limitations
• See SAMPLES FOR ANALYSIS.
• Do not use unsedimented blood samples.
• Do not use hemolyzed blood samples.
• Avoid aged, improperly stored blood samples ; degradation products (or artefacts) may affect the electrophoretic pattern after 7 days storage.
• After 10 days storage, viscous aggregates composed in red blood cells may appear, they must be discarded before analysis.
• When an abnormal hemoglobin is detected, use other means of identification (e.g., globin chain electrophoresis), or consult or send sample to a specialized laboratory.

IMPORTANT: It is also necessary to analyze the hematologic state, as complementary results.
• The migration of a hemoglobin variant close to Hb A involves an underestimation of Hb A fraction and that of the variant and consequently, an overestimation of Hb A2 fraction. In order to quantify Hb A2 with precision, it is necessary to delete the separate integration of both variants and Hb A, and to quantify these fractions together.
• Some homozygous “S” subjects receive a “Hydrea®” (hydroxyurea) treatment that can induce synthesis of foetal hemoglobin. With CAPILLARYS HEMOGLOBIN(E) procedure, the mobility of the induced hemoglobin F is not different from the 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.

Troubleshooting
Call SEBIA Technical Service of the supplier when the test fails to perform while the instruction for the preparation and storage of materials, and for the procedure were carefully followed.
Kit reagent Safety Data Sheets and information on waste products elimination are available from the Technical Service of the supplier.
PERFORMANCE DATA

Results obtained using the CAPILLARYS HEMOGLOBIN(E) procedure indicate a very good reproducibility for quantitative analysis with a mean CV % of about 1.7 % for each hemoglobin component. All electrophoregrams were also interpreted visually. Results presented below have been obtained using the standard parameters of the CAPILLARYS software (smoothing 0 and hemoglobin fractions automatic quantification with HEMOGLOBIN(E) analysis program).

Reproducibility within run
Five (5) different blood samples (normal blood A ; blood B with increased Hb A2 ; bloods C and E with Hb F ; blood F with increased Hb A2 and Hb F and S components) were run in 7 capillaries using the CAPILLARYS HEMOGLOBIN(E) procedure with 2 lots of analysis buffer. The mean, SD and CV (n = 7) were calculated for each sample, each hemoglobin component and each lot. The table shows the values for the 5 tested samples for each hemoglobin component and with the 2 lots of buffer.

Reproducibility between runs
Eight (8) different blood samples were run 10 times using the CAPILLARYS HEMOGLOBIN(E) procedure with three lots of analysis buffer. The samples analyzed included four samples with normal Hb A2 level, and four samples with an abnormal hemoglobin (Hb F or Hb S) and one elevated Hb A2. The mean, SD and CV (n = 10) of different hemoglobin components were calculated for each sample and each lot. The table shows the ranges of hemoglobins values for the 8 tested samples with the three lots of buffer and a mean CV calculated from the pooled CV's for all samples (n = 24).

Reproducibility between lots
Eight (8) different blood samples (identical as those used for “Reproducibility between runs” study) were run 10 times using the CAPILLARYS HEMOGLOBIN(E) procedure with three lots of analysis buffer. The mean, SD and CV (n = 30) of different hemoglobin components were calculated for each sample and each lot. The table shows the ranges of hemoglobins values for the 8 samples tested with the three lots of buffer and a mean CV calculated from the pooled CV's for all samples (n = 3).

In addition, none of the repeats (reproducibility between runs and lots) showed false positive or false negative values.

Linearity
Two blood samples were mixed within different proportions and the dilutions were electrophoresed with CAPILLARYS HEMOGLOBIN(E) procedure. The test was determined to be linear within the entire range studied. In addition, two blood samples from anaemic patients were serially diluted in saline and electrophoresed with CAPILLARYS HEMOGLOBIN(E). The test was determined to be linear within the entire range studied from 2.1 to 12.6 g/dL hemoglobin and hemoglobin fractions percentages were not affected by the hemoglobin concentration of the samples.
Accuracy
NOTE: The blood samples and their diagnostic assessment, used in the three accuracy studies presented below, were provided by hospitals in Europe. The diagnosis was based on HPLC and/or on a routine alkaline gel and acid gel electrophoresis.

Quantitative Determination of Hb A2
The levels of Hb A2 were measured in sixty six (66) blood samples with normal and elevated levels of Hb A2 both by electrophoretic separations obtained with CAPILLARYS HEMOGLOBIN(E) procedure and a commercially available HPLC system for Hb A2 quantification. The measured values of Hb A2 from both procedures were analyzed by a linear regression statistical procedure. The results of linear regression analysis are tabulated below (y = CAPILLARYS HEMOGLOBIN(E)) :

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Correlation coefficient</th>
<th>y-Intercept</th>
<th>Slope</th>
<th>Range of % values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A2</td>
<td>0.947</td>
<td>0.146</td>
<td>0.908</td>
<td>1.6 - 6.0</td>
</tr>
</tbody>
</table>

Quantitative Determination of Hb F
The levels of Hb F were measured in seventy four (74) blood samples with normal and elevated levels of Hb F both by electrophoretic separations obtained with CAPILLARYS HEMOGLOBIN(E) procedure and a commercially available HPLC system for Hb F quantification. The measured values of Hb F from both procedures were analyzed by a linear regression statistical procedure. The results of linear regression analysis are tabulated below (y = CAPILLARYS HEMOGLOBIN(E)) :

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Correlation coefficient</th>
<th>y-Intercept</th>
<th>Slope</th>
<th>Range of % values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb F</td>
<td>0.995</td>
<td>-0.339</td>
<td>0.968</td>
<td>0.0 - 44.9</td>
</tr>
</tbody>
</table>

Quantitative Determination of Hb S
The levels of Hb S were measured in forty three (43) blood samples with normal and elevated levels of Hb S both by electrophoretic separations obtained with CAPILLARYS HEMOGLOBIN(E) procedure and a commercially available HPLC system for Hb S quantification. The measured values of Hb S from both procedures were analyzed by a linear regression statistical procedure. The results of linear regression analysis are tabulated below (y = CAPILLARYS HEMOGLOBIN(E)) :

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Correlation coefficient</th>
<th>y-Intercept</th>
<th>Slope</th>
<th>Range of % values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb S</td>
<td>0.994</td>
<td>1.769</td>
<td>1.005</td>
<td>9.1 - 92.7</td>
</tr>
</tbody>
</table>

Detection of Hemoglobin Abnormalities
Seventy five (75) different blood samples with hemoglobin variants, such as hemoglobins S, C and E, were analyzed with CAPILLARYS HEMOGLOBIN(E) procedure and a commercially available HPLC system for hemoglobins analysis. All abnormal hemoglobins or abnormal levels of normal hemoglobins detected with CAPILLARYS HEMOGLOBIN(E) procedure were in agreement with the comparative HPLC system, hospital results and clinical diagnosis. There were no case observed of false positive, i.e., detection of an abnormal band or abnormal level of a normal band where no such abnormality existed.

BIBLIOGRAPHY

Sang normal
Normal blood sample

Sang bêta-thalassémique
Blood sample with beta-thalassemia

Sang avec variant Hb C
Blood sample with Hb C variant

Sang avec variant hétérozygote Hb S
Blood sample with Hb S heterozygote variant
Figure 5

Hb AHb FHb A2

Sang de bébé (agé de 3 semaines)
Baby blood sample (3 weeks old)

Figure 6

Hb A2

Sang avec Hb F (jeune enfant)
Blood sample with Hb F (young child)

Figure 7

Sang de bébé avec Hb Bart's
Baby blood sample with Hb Bart's

Figure 8

Sang avec Hb H
Blood sample with Hb H
Figure 9

Sang avec variant Hb D-Punjab
Blood sample with Hb D-Punjab variant

Figure 10

Sang avec variant delta Hb A’2
Blood sample with delta Hb A’2 variant

Figure 11

Sang avec variant homozygote Hb E et fraction Hb F élevée
Blood sample with homozygote Hb E variant and elevated Hb F

Figure 12

Sang avec variants hétérozygotes Hb S et Hb C
Blood sample with Hb S & Hb C heterozygote variants
PROFILS ÉLECTROPHORÉTIQUES - ELECTROPHORETIC PATTERNS

Figure 13

Sang avec variant homozygote Hb S (et Hb F)
Blood sample with Hb S homozygote variant (and Hb F)

Figure 14

Sang avec Hb A dégradée (Hb A3) et Hb F faible
Blood sample with degraded Hb A (Hb A3) and faint Hb F

Figure 15

Sang avec variant Hb Lepore
Blood sample with Hb Lepore variant