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 precipitated, centrifuged or washed red blood cells; washing red blood cells is not essential to perform the analysis. 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.

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 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 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 hemoglobinopathies1, 10, 13. 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 abnormalities1, 2, 3, 4, 12. 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 chromatography8, 11.

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 flow9. 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.

By using alkaline pH buffer, normal and abnormal (or variant) hemoglobins are detected in the following order, from cathode to anode: αA2 (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>Hemolysing 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>

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
Before its first use, store the buffer refrigerated (2 to 8 °C). In the original, unopened container, the buffer 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 2 months maximum at room temperature (15 to 30 °C).
When the buffer is not used for a long time, it is recommended to store it refrigerated.
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 Hemolysing Solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or Hemolysing Solution vial label.
Discard Hemolysing Solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.
NOTE: The usual color of hemolysing 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
1. 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.
2. 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.

3. 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.

4. 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.

5. 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.

NOTE: Samples should not be stored at room temperature!
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:
• an additional fraction called Hb A3 (“aging fraction” of Hb A) may be observed more anodic than Hb A on the electrophoretic pattern,
• a weak fraction appears near the Hb S migration zone and,
• when Hb C is present, a fraction appears more anodic than Hb A2 which does not interfere with it.
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.
• 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.

Samples to avoid
• 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 sample-racks;
• Sample hemolysis and dilution from primary tubes (without any plasma) 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 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;
• Setting up the instrument for operation;
• 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 A (Hb A) fraction is automatically identified and adjusted in the middle of the review window.

The resulting electrophoregrams are evaluated visually for pattern abnormalities.

The CAPILLARYS system calculates concentration (%) of each hemoglobin fraction without considering degraded products.

Patterns are automatically adjusted with regard to Hb A fraction to facilitate their interpretation:
- when Hb A is absent (yellow warning signal), the adjustment is performed using the position of the Hb A fraction on the previous electrophoretic pattern obtained with the same capillary,
- when the adjustment is not possible, a red warning signal appears (Call SEBIA).

WARNING: Do not consider hemoglobin A2 quantification when hemoglobin C is present in the blood 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.

RESULTS

Quality control

It is advised to include an assayed control blood (normal blood) or assayed blood sample containing hemoglobins A, F, C and S into each run of samples.

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 : ≥ 96.8 %
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).

Interpretation

See ELECTROPHORETIC PATTERNS.

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. The clinical significance of such a change depends on the type of amino acid and the site involved. In clinically significant disease, either the α-chain or the β-chain is affected.

More than 900 variants of adult hemoglobin have been described. The first abnormal hemoglobin 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) 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); 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 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 is replaced by lysine. With CAPILLARYS HEMOGLOBIN(E) procedure, hemoglobin O-Arab migrates exactly like hemoglobin A2. In such a case, hemoglobin A2 can not be quantified. When this fraction is >15%, hemoglobin O-Arab must be suspected, and is not identified as hemoglobins C and E because they migrate separately from hemoglobin A2.

Hemoglobin D
One glutamic acid of the β-chain is replaced by glutamine. With CAPILLARYS HEMOGLOBIN(E) procedure, hemoglobin D (called D-Punjab, D-Los Angeles, D-Chicago or D-Portugal) 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 tetramer without any α-chain:

• hemoglobin Bart = γ 4,
• hemoglobin H = β 4.

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 F 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 δA’2 (A2 variant), a hemoglobin A2 variant may be suspected.
• When a hemoglobin A2 variant is detected (δA’2 or any other A2 variant), it is recommended to add its percentage to hemoglobin A2 for a better beta-thalassemia diagnostic.

Interference and Limitations

• See SAMPLES FOR ANALYSIS.
• Do not use unsedimented blood samples.
• Avoid aged, improperly stored blood samples: degradation products (or artefacts) may affect the electrophoretic pattern after 7 days storage.
• Do not use hemolyzed blood samples.
• 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, which moves differently than the major hemoglobin variants S, C, E, O-Arab and D, use other means of identification (e.g., globin chain electrophoresis), or consult or send sample to a specialized laboratory.
• 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.

<table>
<thead>
<tr>
<th>Hemoglobin component</th>
<th>Hb A</th>
<th>Hb A2</th>
<th>Hb F</th>
<th>Hb S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood sample A: lot No. 1 / lot No. 2</td>
<td>MEAN (%)</td>
<td>SD</td>
<td>CV (%)</td>
<td>MEAN CV (%)</td>
</tr>
<tr>
<td></td>
<td>97.3 / 97.3</td>
<td>0.05 / 0.05</td>
<td>0.1 / 0.1</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>2.7 / 2.7</td>
<td>0.05 / 0.05</td>
<td>1.8 / 1.8</td>
<td>/</td>
</tr>
<tr>
<td>Blood sample B: lot No. 1 / lot No. 2</td>
<td>MEAN (%)</td>
<td>SD</td>
<td>CV (%)</td>
<td>MEAN CV (%)</td>
</tr>
<tr>
<td></td>
<td>94.3 / 94.5</td>
<td>0.06 / 0.08</td>
<td>0.1 / 0.1</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>5.7 / 5.5</td>
<td>0.06 / 0.08</td>
<td>1.1 / 1.5</td>
<td>/</td>
</tr>
<tr>
<td>Blood sample C: lot No. 1 / lot No. 2</td>
<td>MEAN (%)</td>
<td>SD</td>
<td>CV (%)</td>
<td>MEAN CV (%)</td>
</tr>
<tr>
<td></td>
<td>80.5 / 80.4</td>
<td>0.14 / 0.12</td>
<td>0.2 / 0.1</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>2.3 / 2.3</td>
<td>0.00 / 0.05</td>
<td>0.0 / 2.0</td>
<td>0.8 / 0.5</td>
</tr>
<tr>
<td>Blood sample E: lot No. 1 / lot No. 2</td>
<td>MEAN (%)</td>
<td>SD</td>
<td>CV (%)</td>
<td>MEAN CV (%)</td>
</tr>
<tr>
<td></td>
<td>96.8 / 96.9</td>
<td>0.09 / 0.11</td>
<td>0.1 / 0.1</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>2.4 / 2.3</td>
<td>0.05 / 0.03</td>
<td>1.9 / 1.5</td>
<td>6.7 / 10.2</td>
</tr>
<tr>
<td>Blood sample F: lot No. 1 / lot No. 2</td>
<td>MEAN (%)</td>
<td>SD</td>
<td>CV (%)</td>
<td>MEAN CV (%)</td>
</tr>
<tr>
<td></td>
<td>53.4 / 53.7</td>
<td>0.17 / 0.24</td>
<td>0.3 / 0.4</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>5.0 / 4.9</td>
<td>0.05 / 0.07</td>
<td>1.1 / 1.4</td>
<td>0.6 / 0.8</td>
</tr>
<tr>
<td></td>
<td>9.0 / 8.8</td>
<td>0.05 / 0.07</td>
<td>1.1 / 1.4</td>
<td>0.6 / 0.8</td>
</tr>
</tbody>
</table>

In addition, none of the repeats showed false positive or false negative values.

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).

<table>
<thead>
<tr>
<th>Hb COMPONENT</th>
<th>MEAN (%)</th>
<th>SD</th>
<th>CV (%)</th>
<th>MEAN CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A</td>
<td>53.1 - 97.3</td>
<td>0.00 - 0.85</td>
<td>0.0 - 1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Hb A2</td>
<td>2.4 - 5.0</td>
<td>0.00 - 0.10</td>
<td>0.0 - 1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Hb F</td>
<td>0.7 - 8.7</td>
<td>0.04 - 0.07</td>
<td>0.7 - 8.8</td>
<td>4.5</td>
</tr>
<tr>
<td>Hb S</td>
<td>30.8 - 42.7</td>
<td>0.19 - 0.83</td>
<td>0.5 - 1.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

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

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).

<table>
<thead>
<tr>
<th>Hb COMPONENT</th>
<th>MEAN (%)</th>
<th>SD</th>
<th>CV (%)</th>
<th>MEAN CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A</td>
<td>53.2 - 97.3</td>
<td>0.04 - 0.77</td>
<td>0.04 - 1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Hb A2</td>
<td>2.4 - 5.0</td>
<td>0.03 - 0.09</td>
<td>1.0 - 2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Hb F</td>
<td>0.7 - 8.6</td>
<td>0.06 - 0.08</td>
<td>0.9 - 8.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Hb S</td>
<td>31.1 - 42.0</td>
<td>0.29 - 0.71</td>
<td>0.9 - 1.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

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 a hospital. 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 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 hemoglobin 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

Figure 1
Hb A
Hb A2
Sang normal
Normal blood sample

Figure 2
Hb A
Hb A2
Sang bêta-thalassémique
Blood sample with beta-thalassemia

Figure 3
Hb A
Hb C
Hb A2
Sang avec variant Hb C
Blood sample with Hb C variant

Figure 4
Hb A
Hb D-Punjab
Hb A2
Sang avec Hb D-Punjab
Blood sample with Hb D-Punjab
Figure 5

Sang de bébé (agé de 3 semaines)
Blood sample from baby (3 weeks old)

Figure 6

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

Figure 7

Sang avec variant Hb H
Blood sample with Hb H variant

Figure 8

Sang avec variant hétérozygote Hb S
Blood sample with Hb S heterozygote variant
**SCHÉMAS / FIGURES**

**PROFILS ÉLECTROPHORÉTIQUES - ELECTROPHORETIC PATTERNS**

**Figure 9**

![Hb A, Hb A2, Hb delta A']

Sang avec variant delta Hb A'
Blood sample with delta Hb A' variant

**Figure 10**

![Hb E, Hb F, Hb A2]

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

**Figure 11**

![Hb S, Hb C]

Sang avec variants hétérozygotes Hb S et Hb C
Blood sample with Hb S & Hb C heterozygote variants

**Figure 12**

![Hb S, Hb F, Hb A2]

Sang avec variant homozygote Hb S (et Hb F)
Blood sample with Hb S homozygote variant (and Hb F)
Sang dégradé avec Hb A3

Degradated blood sample with Hb A3