CAPILLARYS Hb A1c
USING THE CAPILLARYS 2 FLEX-PIERCING INSTRUMENT
INTENDED USE

The CAPILLARYS Hb A1c kit is designed for separation and quantification of the HbA_1c glycated fraction of hemoglobin in human blood, by capillary electrophoresis in alkaline buffer (pH 9.4) with the CAPILLARYS 2 FLEX-PIERCING instrument. Measurement of hemoglobin A_1c is effective in monitoring long-term glycemic control in individuals with diabetes mellitus. The CAPILLARYS Hb A1c kit is designed for Professional Use Only.

For In Vitro Use.

PRINCIPLE OF THE TEST 1-29

Hemoglobin glycation is a non-enzymatic reaction between the intra-erythrocyte glucose and the N-terminal amino-group of the hemoglobin ß chains. This reaction takes place during the entire life of the red blood cells. The rate of glycated hemoglobin formation is related to the glycemia insofar as the intra-erythrocyte glucose concentration does not depend on insulin but only on the glycemia. It accumulates in red blood cells during the 120 days of their life [1, 9, 15, 21, 23].

The level of glycated hemoglobin corresponds to the "integration" of all the glycemic variations during the previous weeks. It can be used as an index of diabetes control. This quantification allows to evaluate the middle term efficiency of treatments [11, 12, 14, 16, 18, 19, 22, 25, 29].

Electrophoresis is a well established technique routinely used in clinical laboratories for measuring components from body fluids, including HbA_1c glycated fraction [13, 17, 20]. The CAPILLARYS 2 FLEX-PIERCING instrument has been developed to provide complete automation of this testing with fast separation and good resolution. In many aspects, the methodology can be considered as an intermediary type of technique between classical zone electrophoresis and liquid chromatography.

The CAPILLARYS 2 FLEX-PIERCING instrument 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 2 FLEX-PIERCING instrument has silica capillaries functioning in parallel allowing 8 simultaneous analyses for HbA_1c quantification from whole blood sample. 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 the cathodic end of the capillary at 415 nm, which is the absorbance wave length specific to hemoglobins. Before each run, the capillaries are washed with a wash solution and prepared for the next analysis with buffer. Direct detection provides accurate relative quantification of individual hemoglobin A_1c fraction.

In addition, the high resolution of CAPILLARYS Hb A1c procedure allows the quantification of HbA_1c and particularly, even in the presence of labile HbA_1c, carbamylated and acetylated hemoglobins, and major hemoglobin variants.

By using alkaline pH buffer, normal and abnormal (or variant) hemoglobins are detected in the following order, from cathode to anode : A2/C, E, S/D, F, A0, other Hb (including minor Hb A1) and then A_1c.

REAGENTS AND MATERIALS SUPPLIED IN THE CAPILLARYS Hb A1c KIT

WARNING : See the safety data sheets.

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 2015</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, 700 mL</td>
</tr>
<tr>
<td>Wash solution (stock solution)</td>
<td>1 vial, 75 mL</td>
</tr>
<tr>
<td>Dilution segments</td>
<td>1 pack of 90</td>
</tr>
<tr>
<td>Filters</td>
<td>4 filters</td>
</tr>
</tbody>
</table>

During transportation, the kit can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

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.

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

1. BUFFER
Preparation
The buffer is ready to use. It contains : buffer solution pH 9.4 ± 0.5 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
Buffer for analysis of HbA_1c with capillary electrophoresis.

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.

IMPORTANT : When stored at 2 – 8 °C and prior to use, it is necessary for the buffer to reach room temperature; when it is full, let the buffer vial at room temperature for at least 3 hours prior to use. If this precaution is not respected, the performances of the procedure may be affected.

WARNING : Do not pre-heat the buffer in hot water.
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.

If the buffer vial is planned to be used within 20 days, it may be stored at room temperature. Once the buffer vial has been opened and positioned on the CAPILLARYS 2 FLEX-PIERCING instrument, it is stable for a maximum of 20 days (accumulated) at room temperature (15 to 30 °C).

IMPORTANT : The accumulated time of the buffer stored at room temperature must not exceed 20 days. This time of 20 day storage takes account of the time for the buffer to come to room temperature.

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

NOTE : During storage, the buffer may present a slight color without any adverse effects on its performance.

2. HEMOLYSING SOLUTION

Preparation
Hemolysing Solution is ready to use. It contains: components, nonhazardous at the concentration used, necessary for optimum performance.

Use
To dilute and hemolyze whole blood.

Storage, stability and signs of deterioration
Store hemolysing solution at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). It is stable until the expiration date indicated on the kit package or hemolysing solution vial label. DO NOT FREEZE.

Discard hemolysing solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

NOTE : During storage, hemolysing solution may turn yellow without any adverse effects on its performance.

3. WASH SOLUTION

Preparation
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.

Use
For washing the capillaries after HbA1c electrophoresis.

IMPORTANT : Before filling the wash solution container, it is recommended to wash the opening of the container, the connector and the tube with plenty of distilled or deionized water to avoid salts deposit.

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 Hb A1c procedure.

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

5. FILTERS

Use
Disposable filters for filtration of analysis buffer, hemolysing solution, working wash solution and distilled or deionized water (used for capillaries rinsing).

IMPORTANT : When kit replacement, change systematically all the filters.

Screw one filter at the connector situated at the extremity of each tube that plunges in the vials of buffer, hemolysing solution, wash solution and distilled or deionized water. When setting filters on CAPILLARYS 2 FLEX-PIERCING instrument, rinse the connectors and the tubes with distilled or deionized water.

Storage
Before use, store the filters in their sealed package in a dry place at room temperature or refrigerated.

REAGENTS REQUIRED BUT NOT SUPPLIED

WARNING : See the safety data sheets.

1. Hb A1c CAPILLARY CALIBRATORS

Composition
Hb A1c CAPILLARY Calibrators (SEBIA, PN 4755) are obtained from pools of human blood samples. They contain stabilizers and preservatives to maintain the stability of the hemoglobin fractions. The calibrators are in a stabilized lyophilized form.

Hb A1c CAPILLARY Calibrator 1 presents a normal HbA1c level and Hb A1c CAPILLARY Calibrator 2 presents an elevated HbA1c level.

Intended use
The Hb A1c CAPILLARY Calibrators 1 and 2 are designed for the calibration and migration control of human glycated hemoglobin A1c quantification with CAPILLARYS Hb A1c electrophoresis procedure performed with the CAPILLARYS 2 FLEX-PIERCING automated instrument for capillary electrophoresis, in order to achieve results in patient blood samples that are comparable to the DCCT study and traceable to the IFCC reference system.
The recommendations to calibrate are the following:

- Perform 3 successive series of analyses with both calibrators:
  - for the first use of the "Hb A1c" analysis program with the CAPILLARYS 2 FLEX-PIERCING instrument;
  - after having changed a capillary;
  - after having changed the lot number of calibrators.
- Perform 1 series of analyses with both calibrators, and then with 1 of the 2 controls, before starting a new analysis sequence:
  - after having changed the lot number of analysis buffer;
  - after technical operation;
  - in case of analyses of controls giving HbA1c values outside the expected values (and after having confirmed this deviation by the analysis of a second dilution segment with blood control);
  - at least every 2 months.

**NOTE:** It is not necessary to calibrate the instrument after having changed the lot number of hemolyzing solution.

**IMPORTANT:** For optimal use of each Hb A1c CAPILLARY Calibrator with the CAPILLARYS 2 FLEX-PIERCING instrument, it is necessary to use one specific tube designed for blood controls and its corresponding cap (see "EQUIPMENT AND ACCESSORIES REQUIRED", Tubes and caps for Controls) and to identify this tube with the corresponding calibrator bar code label.

- Reconstitute each lyophilized Hb A1c CAPILLARY Calibrator 1 and 2 vial with the volume of distilled or deionized water and according to the procedure indicated in the package insert of the Hb A1c CAPILLARY Calibrators. Mix gently the calibrator vial to dissolve the whole lyophilized blood, ensure that no liquid contacts the cap. Allow to stand for 30 minutes at 2 – 8 °C and mix gently (avoid formation of foam).

**NOTE:** The precision of the reconstitution volume to be maintained is ± 1.0 %.
- Apply each reconstituted calibrator in a tube designed for blood control.
  - Close the tube with its cap.
  - For each calibrator, place a wedge adapter for the blood control tube in position No. 1 on a CAPILLARYS 2 FLEX-PIERCING sample rack No. F0 intended for control blood sample, containing a new green dilution segment.
- Place the tube with the calibrator (identified with the specific bar code label of this calibrator) on the wedge adapter on the sample rack No. F0.
- Start the analysis: Slide the sample rack No. F0 into the CAPILLARYS 2 FLEX-PIERCING instrument and enter in the window which appears on the screen the parameters of the analyzed calibrator, indicated in the package insert of the Hb A1c CAPILLARY Calibrators: HbA1c level in mmol/mol, lot number and expiration date.

**NOTE:** HbA1c concentration is indicated in IFCC unit (mmol/mol).
- Select "Automatic dilution" in the same window and validate.
- The results are then automatically considered by the software for the data analysis.

**IMPORTANT:** For optimal use of each Hb A1c CAPILLARY Calibrator, it is necessary to use one bar code label intended to identify the tube for control which contains the calibrator (close the tube with its specific cap before using it). The software displays the HbA1c value for both calibrators that has been entered by the operator.

**NOTE:** Both calibrators must imperatively be analyzed for an effective calibration: the run order of both calibrators is indifferent. As soon as it is ejected and within 10 minutes maximum, the dilution segment with each hemolyzed calibrator may be analyzed again by sliding the corresponding rack into the CAPILLARYS 2 FLEX-PIERCING instrument (let the tube identified with the bar code label on the sample rack). During the second analysis of this dilution segment, in the "Hb A1c Calibrator" window which appears on the screen, select "Manual dilution" and validate. The dilution segment with a hemolyzed calibrator can be re-used only once.

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

This wedge adapter is intended to support the conical tubes for blood controls (or calibrators) on a sample rack No. F0 or on a rack for samples of the CAPILLARYS 2 FLEX-PIERCING instrument. It presents 2 markers which allow estimating of the volume of blood control (or calibrator) available to perform the analysis:

- 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 250 µL of blood control (or calibrator) in the tube. When the volume of blood control (or calibrator) reaches this level or is higher, it is sufficient to perform the complete analysis of this blood with the sample rack No. F0.
- when the tube is supported by the wedge adapter, the lower level is located at the bottom of the crenellations and corresponds to a volume of about 100 µL of blood control (or calibrator) in the tube. When the volume of blood control (or calibrator) reaches this level or is comprised between the 2 markers of the wedge adapter, it is sufficient to perform one analysis of this blood on a sample rack.

**Storage, stability and signs of deterioration**

*See the package insert of the Hb A1c CAPILLARY Calibrators.*

**WARNING:** No test method can provide an absolute assurance of the absence of HIV, hepatitis B and C or other infectious agents. Therefore, handle the Hb A1c CAPILLARY Calibrators as a hazardous biological material.

These lots of bloods were found negative on assays approved by FDA or EU equivalent regulatory agency:

- against hepatitis B surface antigen;
- for antibody to HCV;
- for antibody to HIV1 and HIV2.

### 2. Hb A1c CAPILLARY CONTROLS

#### Composition

Hb A1c CAPILLARY Controls (SEBIA, PN 4774) are obtained from pools of human blood samples. They contain stabilizers and preservatives to maintain the stability of the hemoglobin fractions. The controls are in a stabilized lyophilized form.

Hb A1c CAPILLARY Control 1 presents a normal HbA1c level and Hb A1c CAPILLARY Control 2 presents an elevated HbA1c level.

**Start the analysis:** Slide the sample rack No. F0 into the CAPILLARYS 2 FLEX-PIERCING instrument and enter in the window which appears on the screen the parameters of the analyzed calibrator, indicated in the package insert of the Hb A1c CAPILLARY Calibrators: HbA1c level in mmol/mol, lot number and expiration date.
Intended use
The Hb A1c CAPILLARY Controls 1 and 2 are designed for the quality control of human glycated hemoglobin A1c quantification with CAPILLARYS Hb A1c electrophoresis procedure performed with the CAPILLARYS 2 FLEX-PIERCING automated instrument for capillary electrophoresis. The values obtained must fall within the range determined for each batch.

IMPORTANT: For optimal use of each Hb A1c CAPILLARY Control with the CAPILLARYS 2 FLEX-PIERCING instrument, it is necessary to use one specific tube designed for blood controls and its corresponding cap (see "EQUIPMENT AND ACCESSORIES REQUIRED", Tubes and caps for Controls) and to identify this tube with the corresponding control bar code label.

Determination of customized values for Hb A1c CAPILLARY Controls:
Each laboratory must establish values for Hb A1c CAPILLARY Controls 1 and 2 that are specific for each CAPILLARYS 2 FLEX-PIERCING automated instrument according to the procedure indicated in the package insert of the Hb A1c CAPILLARY Controls.

WARNING: The determination of controls values must always be performed:
- after the first calibration of the CAPILLARYS 2 FLEX-PIERCING instrument;
- after having changed one or many capillaries;
- after having changed the lot number of calibrators or controls.
See the package insert of the Hb A1c CAPILLARY Controls.

Quality control:
The recommendations to analyze one of the two controls on whole 8 capillaries are the following:
- after capillaries activation;
- after each calibration of the instrument performed with the Hb A1c CAPILLARY Calibrators;
- after a capillary cleaning sequence with CAPICLEAN;
- before starting a new analysis sequence;
- every 10 sample racks, by alternating Hb A1c CAPILLARY Control 1 and Control 2 for high-volume testing laboratories.

- Reconstitute each lyophilized Hb A1c CAPILLARY Control 1 and 2 vial with the volume of distilled or deionized water indicated in the package insert of the Hb A1c CAPILLARY Controls. Allow to stand for 30 minutes and mix gently (avoid formation of foam).

NOTE: The precision of the reconstitution volume to be maintained is ± 1.0 %.
- Apply each reconstituted control in a tube designed for blood control.
- Close the tube with its cap.
- Place each tube with the control (identified with its specific bar code label) on a wedge adapter for the blood control tubes, in position No. 1 on a CAPILLARYS 2 FLEX-PIERCING sample rack No. F0 intended for blood control samples, containing a new dilution segment.

NOTE: In order to avoid any confusion, it is recommended to use a white dilution segment for Hb A1c CAPILLARY Control 1 and a grey dilution segment for Hb A1c CAPILLARY Control 2.
- Start the analysis: Slide the sample rack into the CAPILLARYS 2 FLEX-PIERCING instrument.
- The results are then automatically considered by the software for the data analysis.
- Check the concentration levels and percentages for HbA1c fraction obtained from the analyses of controls with established customized values of the instrument. They must fall within the range determined for each batch. If not, calibrate again the instrument with the Hb A1c CAPILLARY Calibrators (see § "Hb A1c CAPILLARY CALIBRATORS").

NOTE: HbA1c concentration displayed by the software is indicated in mmol/mol, without any decimal place according to IFCC recommendations. This decimal place is however considered for the characterization of the sample (as normal sample or sample with elevated HbA1c level), statistics and Levey Jennings charts.

IMPORTANT: For optimal use of each Hb A1c CAPILLARY Control, it is necessary to use one bar code label intended to identify the tube for control which contains the blood control (close the tube with its specific cap before using it).

Storage, stability and signs of deterioration
See the package insert of the Hb A1c CAPILLARY Controls 1 and 2.

NOTE: It is recommended to store dilution segments with hemolyzed Hb A1c CAPILLARY Controls 1 and 2 in boxes for controls storage (see "EQUIPMENT AND ACCESSORIES REQUIRED", Boxes for controls storage).

WARNING: No test method can provide an absolute assurance of the absence of HIV, hepatitis B and C or other infectious agents. Therefore, handle the Hb A1c CAPILLARY Controls as a hazardous biological material.
These lots of bloods were found negative on assays approved by FDA or EU equivalent regulatory agency:
- against hepatitis B surface antigen;
- for antibody to HCV;
- for antibody to HIV1 and HIV2.

3. DISTILLED OR DEIONIZED WATER
Use
For rinsing capillaries in automated instrument CAPILLARYS 2 FLEX-PIERCING, 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.
For optimal operation, add 35 μL/dL of CLEAN PROTECT (SEBIA, PN 2059, 1 vial of 5 mL).

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

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

Use
For sample probe cleaning in automated instrument CAPILLARYS 2 FLEX-PIERCING, SEBIA, for capillary electrophoresis, during the CAPICLEAN cleaning sequence.

IMPORTANT: Launch a CAPICLEAN cleaning sequence at least once a week and at maximum once a day, or after every 500 analyses when performed within less than one week.

See the instruction sheets of CAPICLEAN, SEBIA.

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. Precipitate or combined particles in suspension (flocules) may be observed in the CAPICLEAN vial without any adverse effects on its utilization. Do not dissolve this precipitate or these particles. It is recommended to collect only the supernatant.

5. SODIUM HYPOCHLORITE SOLUTION (for sample probe cleaning)

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

Use
For the sample probe cleaning in the CAPILLARYS 2 FLEX-PIERCING Instrument, SEBIA (weekly maintenance in order to eliminate adsorbed proteins from the probe).

See the CAPILLARYS 2 FLEX-PIERCING instruction manual, 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 2 FLEX-PIERCING Instrument.
• In the "MAINTENANCE" window which appears on the screen, select "Launch the 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 3 months. Avoid storage in sunlight, close to heat and ignition source, and to acids and ammonia.

6. CAPILLARYS / MINICAP WASH SOLUTION

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

Use
For washing the capillaries of CAPILLARYS 2 FLEX-PIERCING. This additional reagent is needed when the number of tests in series is below 40.

IMPORTANT: Before filling the wash solution container, it is recommended to wash the opening of the container, the connector and the tube with plenty of distilled or deionized water to avoid salts deposit.

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.

NOTES:
The assays that were performed for the validation of reagents demonstrated that, for the different solutions and using an adapted equipment for the reconstitution volume, a variation of ± 5 % on the final volume has no adverse effect on the analysis. The distilled or deionized water used to reconstitute solutions, must be free of bacterial proliferation and mold (use a 0.22 µm filter) and have a resistivity higher than 10 Megohms x cm.

EQUIPMENT AND ACCESSORIES REQUIRED

1. CAPILLARYS 2 FLEX-PIERCING Instrument SEBIA, PN 1227.
2. Sample racks supplied with CAPILLARYS 2 FLEX-PIERCING.
3. CAPILLARYS 2 FLEX-PIERCING racks for tubes 11 mm, SEBIA, PN 1360, 5 units.
4. Container Kit supplied with CAPILLARYS 2 FLEX-PIERCING: Rinse (to fill with distilled or deionized water), wash solution and waste container.
5. Collection tubes with 13 mm diameter and their corresponding caps (maximal length of tube with cap: 90 mm, maximal diameter of cap: 17 mm): for example, BD Vacutainer, Terumo Venosafe 5 mL, Greiner Bio-one Vacuette 1, 2, 3 or 4 mL or Sarstedt S-Monovette 4 mL tubes (13 x 75 mm), or collection tubes with 11 mm diameter and their corresponding caps (maximal length of tube with cap: 90 mm, maximal diameter of cap: 17 mm): for example, Sarstedt S-Monovette 2,7 mL or Kabe Laborteknik Primavette S 2,6 mL tubes (11 x 66 mm), or collection tubes with equivalent dimensions approved for clinical assays.
6. Tubes and caps for Controls, SEBIA, PN 9205: 500 conical tubes and their caps to analyze calibrators and blood controls with the CAPILLARYS 2 FLEX-PIERCING instrument.
7. Wedge adapters for tubes for controls, SEBIA, PN 9203, 10 units (or supplied with CAPILLARYS 2 FLEX-PIERCING).
8. Boxes for controls storage, SEBIA, PN 2082: 2 boxes for storage of dilution segments containing hemolyzed Hb A1c CAPILLARY Controls 1 and 2.

SAMPLES FOR ANALYSIS

Sample collection and storage
Fresh anticoagulated whole blood samples collected in tubes containing K$_2$EDTA or K$_3$EDTA as anticoagulant are recommended for analysis. Blood must be collected according to established procedures used in clinical laboratory testing.

Samples can be stored for 7 days maximum between 2 and 8 °C or 18 hours maximum at room temperature (between 15 and 30 °C). For longer storage, samples can be frozen at – 80 °C within 8 hours of collection without any preparation.

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.

Sample preparation

• Use directly whole blood samples.
• Check that all the tubes contain 1 mL minimum of blood and are perfectly closed.
• Vortex for 5 seconds blood samples stored at 2 – 8 °C for one week or stored at – 80 °C.

WARNING: The tubes must be closed with their corresponding caps designed for the CAPILLARYS Hb A1c procedure with the CAPILLARYS 2 FLEX-PIERCING instrument (see EQUIPMENT AND ACCESSORIES REQUIRED).

Particular case:

Analysis of samples with volume below 1 mL:
- Vortex for 5 seconds the whole blood sample.
- Apply in a conical tube for control at least 100 µL of whole blood to analyze and cap the tube.
- Place the tube with a wedge adapter on a sample rack of the CAPILLARYS 2 FLEX-PIERCING instrument.
- Slide the sample rack into the CAPILLARYS 2 FLEX-PIERCING instrument at the beginning of an analysis series.
- Perform the analysis of this sample according to the standard procedure like a usual blood sample without any delay.

NOTES: It is recommended to gather samples with volume below 1 mL on the same sample rack and analyze them at the beginning of an analysis series. Mix well the sample applied in a conical tube for the analysis before sliding the sample rack into the automated instrument. Without any bar code label on the conical tube, the sample cannot be identified.

Samples to avoid

• Avoid coagulated 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: an additional fraction may migrate particularly to Hb A2 position or more anodically than Hb A0 (in the "other Hb A" position) when analyzing such samples.

In these 2 previous cases, aggregates in red blood cells may affect the collection of the sample by the probe.
• Do not analyze directly tubes containing less than 1 mL of blood sample, the analysis should be affected (see particular case).

PROCEDURE

The CAPILLARYS 2 FLEX-PIERCING instrument is a multiparameter instrument for hemoglobins analysis on parallel capillaries. The hemoglobins assay uses 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 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.

PLEASE CAREFULLY READ THE CAPILLARYS 2 FLEX-PIERCING INSTRUCTION MANUAL.

I. PREPARATION OF CAPILLARYS ANALYSIS

1. Switch on CAPILLARYS 2 FLEX-PIERCING instrument and computer.
2. Set up the software, enter and the instrument automatically starts.
3. The CAPILLARYS Hb A1c kit is intended to run with "Hb A1c" analysis program from the CAPILLARYS 2 FLEX-PIERCING instrument. To select "Hb A1c" analysis program and place the CAPILLARYS Hb A1c buffer and hemolyzing solution vials in the instrument, please read carefully the CAPILLARYS 2 FLEX-PIERCING instruction manual.
4. The sample rack contains 8 positions for sample tubes. Place up to 8 capped sample tubes with whole blood on each sample rack (positions 1 to 8); the bar code of each tube must be visible in the openings of the sample rack.
II. RESULT ANALYSIS  

DILUTION - MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS  

1. Bar codes are read on both sample tubes and sample racks.  
2. Mixing of tubes.  
3. Samples are diluted in hemolysing solution and the sample probe is rinsed after each sample.  
4. Capillaries are washed.  
5. Diluted samples are injected into capillaries.  
6. Migration is carried out under constant voltage for about 9 minutes and the temperature is controlled by Peltier effect.  
7. Hemoglobins are detected directly by scanning at 415 nm and an electrophoretic profile appears on the screen of the instrument.  

NOTE: As the sample is characterized as normal or with elevated HbA1c level using the real value of HbA1c concentration in mmol/mol (whole number calculated by the software for the data analysis), a discordance may appear for HbA1c levels close to the threshold value.  

The identification of normal blood samples and of blood samples with elevated HbA1c level is automatically performed and the profiles can be distinguished in the curve review window of patterns by a blue color for normal samples and a orange color for samples with elevated HbA1c level:  
- Normal blood samples, with "normal" HbA1c concentration lower than 42 mmol/mol (6.0 %) or equal are indicated in blue color.  
- Blood samples with elevated HbA1c concentration, higher than 42 mmol/mol (6.0 %), are indicated in orange color.  

Electrophoretic patterns with abnormality (such as an additional fraction or deletion of a normal fraction among HbA1c, Other Hb A, Hb A0 and Hb A2 fractions) are indicated in purple color with "Atypical profile" and "Hb A1c (*)" indications.  

Patterns are automatically adjusted with regard to Hb A0 fraction to facilitate their interpretation.  

The following table presents the warning and message signals that are displayed and the procedures to follow according to the analyzed sample:  

<table>
<thead>
<tr>
<th>Analyzed sample</th>
<th>Calibrators identified with bar code labels</th>
<th>Controls identified with bar code labels</th>
<th>Blood sample from patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Warning signal: HbA1c value outside specifications for calibrators</td>
<td>Warning message: &quot;analysis of the control not in conformity&quot;: analyze a control blood with a sample rack F0 or repeat the calibration [in case of invalid calibration on one (or many) capillary(ies), the capillary(ies) is(are) deactivated, the HbA1c level is then not displayed].</td>
<td>Warning message: &quot;too low OD&quot; (&lt; 0.10) for a blood sample that is not abnormal, repeat the analysis: if the result is confirmed, the HbA1c level can be reported.</td>
</tr>
<tr>
<td></td>
<td>No detection of Hb A0 and / or HbA1c fraction</td>
<td>No HbA1c value displayed</td>
<td>&quot;Atypical profile&quot; and &quot;HbA1c (*)&quot;: suspect the presence of a Hb variant.</td>
</tr>
<tr>
<td></td>
<td>Insufficient optical density for Hb A0 fraction</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>&quot;Atypical profile&quot; (presence of an additional fraction or deletion of a normal fraction)</td>
<td>With the Quality Control mode: &quot;+&quot; or &quot;+&quot;, identification according to the HbA1c level compared to the customized values entered by the operator.</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>HbA1c value outside the expected values for controls analyzed with the Quality Control (QC) mode</td>
<td>With the Quality Control mode, if the warning message &quot;analysis of the control not in conformity&quot; is displayed, repeat the calibration.</td>
<td>/</td>
</tr>
</tbody>
</table>

WARNING: Dilution segments with biological samples have to be handled with care.
When a sample has a Hb A2 percentage higher than 3.0 %, an exclamation mark is displayed near the name of the fraction ("Hb A2 !"). Then, a beta thalassemia syndrome, that could affect the HbA1c synthesis, may be suspected (case of physio-pathological interference). It is recommended to analyze the sample with the CAPILLARYS HEMOGLOBIN(E) procedure to verify the Hb A2 percentage and to study the patient's clinical data. However, the HbA1c quantification represents a useful relative follow up index for the same patient.

PLEASE CAREFULLY READ THE CAPILLARYS 2 FLEX-PIERCING INSTRUCTION MANUAL.

III. 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 instrument in order to store capillaries in optimal conditions.

IV. FILLING OF REAGENT CONTAINERS

The CAPILLARYS 2 FLEX-PIERCING instrument 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;
• Place a new hemolysing solution 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.

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

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

PLEASE CAREFULLY READ THE CAPILLARYS 2 FLEX-PIERCING INSTRUCTION MANUAL.

QUALITY CONTROL

After capillaries activation, after each calibration of the instrument performed with the Hb A1c CAPILLARY Calibrators, after a capillary cleaning sequence with CAPICLEAN and before starting a new analysis sequence, it is necessary to run analyses with Hb A1c CAPILLARY Controls 1 or 2, SEBIA, PN 4774.

In addition, for high-volume testing laboratories, it is advised to analyze one of the two controls every 10 sample racks, by alternating Hb A1c CAPILLARY Control 1 and Control 2.

WARNING : When controls values fall out of the customized values range, the samples analyzed on the affected capillary(ies), since the last validated quality control, must be analyzed again.

IMPORTANT : For optimal use of the blood controls analyzed with the CAPILLARYS 2 FLEX-PIERCING instrument, it is necessary to use the specific conical tubes for controls and their corresponding caps, the wedge adapters for tubes for controls (see "EQUIPMENT AND ACCESSORIES REQUIRED") and the bar code labels intended to identify the tubes for controls that contain the blood control to analyze (see the paragraph "Hb A1c CAPILLARY Calibrators" for the utilization of a wedge adapter for tubes for controls).

* US customers : Follow federal, state and local guidelines for quality control.

RESULTS

The CAPILLARYS Hb A1c procedure performed with the CAPILLARYS 2 FLEX-PIERCING instrument has been certified by the National Glycohemoglobin Standardization Program (NGSP).

Values

Direct detection at 415 nm in capillaries yields relative concentrations (percentages) of individual hemoglobin zones, and specially the calibrated HbA1c concentration.

Hemoglobin A1c expected value range was cited from the American Diabetes Association (Standards of Medical Care in Diabetes 2012, 35 (Suppl 1) S11 – S63).

The goal for diabetic patients is to keep the HbA1c level below 6.5 – 7.0 % (i.e., 48 – 53 mmol/mol).

WARNING : Normal value for HbA1c must be considered only when hemoglobin variants are absent.

Actually, according to IFCC and NGSP recommendations, this value has been established for individuals without any hemoglobinopathy. For an atypical sample (with Hb variant), normal value for HbA1c is not displayed by the software but HbA1c quantitative determination represents a useful relative follow up index for the same patient.

Interpretation

See ELECTROPHORETIC PATTERNS.

Interferences

NOTE : The common interfering factors with the CAPILLARYS Hb A1c procedure (triglycerides, bilirubin, ascorbic acid, urea, rheumatoid factor, glybenclamide, acetylated and carbamylated hemoglobins, labile HbA1c, and Hb F) 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 interference with the CAPILLARYS Hb A1c procedure was detected due to the blood sample’s high concentration of the following interfering factors tested at levels equal to the concentrations listed below:

<table>
<thead>
<tr>
<th>Interfering factor</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>1.12 g/dL (12.8 mM)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>25.6 mg/dL (438 µM)</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>60 mg/dL (3.41 mM)</td>
</tr>
<tr>
<td>Urea</td>
<td>291 mg/dL (48.5 mM)</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>2178 IU/mL</td>
</tr>
<tr>
<td>Glybenclamide</td>
<td>3 mg/dL</td>
</tr>
</tbody>
</table>

- No interference with the CAPILLARYS Hb A1c procedure was detected due to the presence of carbamylated hemoglobin (≤ 8.1 %) and labile HbA1c (≤ 10.5 %).
- Acetylated hemoglobin may migrate in minor hemoglobins migration zone, no interference has been observed with HbA1c fraction quantification due to the presence of acetylated hemoglobin (≤ 9.0 %).
- Levels of Hb F up to 15 % in the blood sample do not interfere with HbA1c fraction quantification. Samples that contain high amounts of Hb F (> 15 %), usually found in some people with thalassemia, infants, and some pregnant women, may yield a lower than expected HbA1c result with this assay.
- Analysis with hemoglobin variants:
  - No interference has been observed with HbA1c fraction quantification due to the presence of major abnormal hemoglobins Hb S (≤ 40 %), Hb C (≤ 44 %), Hb D (≤ 41 %) and Hb E (≤ 32 %) (except Bart’s hemoglobin). However, due to the number of variants, the presence of another hemoglobin variant may be observed in the HbA1c migration zone; in the case of a shoulder on HbA1c, no result will be reported by the software.
  - Glycated forms of common hemoglobin variants (Hb S, Hb C, Hb D or Hb E for example) co-migrate with Hb A0 fraction or minor Hb A1 fractions (“other Hb A” fraction) without any modification of the HbA1c result.
  - Some hemoglobin variants may appear as a shoulder of Hb A0 fraction that may not be detected by the software. Only a visual examination of the electrophoretic pattern allows the detection of this shoulder. Do not report any HbA1c result in that case. It is necessary to analyze the hematologic state and to perform complementary studies in order to confirm the presence of a variant.
  - In addition, among variants which migrate close to Hb A0, or joined with Hb A0, some of them may show an additional fraction (“X1c”) that migrates separately from HbA1c. The electrophoretic pattern will be indentified as “Atypical profile”. Do not report any HbA1c result in that case.
  - When analyzing samples without any Hb A (from homozygous patients or with heterozygous variants S/S or S/C, for example) and when Hb F is present, it may be confused with Hb A0 due to their similar migration positions. No HbA1c result will be reported by the software due to the absence of HbA1c in this kind of sample.
- Individuals with recent significant blood loss exhibit falsely low HbA1c values due to a higher fraction of young erythrocytes.
- Abnormal life span of red blood cells, as found in hemolytic anemias, polycythemia or post splenectomy, may affect the levels of HbA1c. However, the values represent a useful relative follow up index for the same patient.

Limitations
- See SAMPLES FOR ANALYSIS.
- Analyze only blood samples contained in collection tubes indicated in the paragraph “EQUIPMENT AND ACCESSORIES REQUIRED” or tubes with equivalent dimensions approved for clinical assays. Call SEBIA technical service for further information on these devices.
- Do not analyze directly tubes containing less than 1 mL of blood sample.
- Avoid aged, improperly stored blood samples; degradation products (or artefacts) may affect the electrophoretic pattern after 7 days storage. When analyzing such samples, an additional fraction may migrate particularly to Hb A2 position or more anodically than Hb A0 (in the “other Hb A” position).
- After 10 days storage, viscous aggregates composed in red blood cells may appear, they must be discarded before analysis.
- In some blood samples from A / C heterozygous patients with Hb F, the Hb A0 fraction may be quantified with imprecision.
- Due to the resolution and sensitivity limits of zone electrophoresis, it is possible that HbA1c may not be quantified in presence of all hemoglobin variants 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 cleaning and waste disposal, labeling and safety rules applied by SEBIA, packaging for the transportation of biological samples, and instruments cleaning are available on the “INSTRUCTIONS & SAFETY DATA SHEETS” DVD.

PERFORMANCE DATA

Precision
The precision of the CAPILLARYS Hb A1c procedure was evaluated in a study based on the Clinical Laboratory Standards Institute (CLSI - USA) EP5-A2 guideline “Evaluation of Precision Performance of Quantitative Measurements Methods”. The means, standard deviations and coefficients of variation (CV's %) (n = 80) were calculated for HbA1c concentration (mmol/mol) and percentage (%) for each sample, using statistical tools recommended by CLSI.
Reproducibility within the same capillary

Eight (8) different blood samples were run using the CAPILLARYS Hb A1c procedure in 8 capillaries of the same CAPILLARYS 2 FLEX-PIERCING instrument and with 1 lot of CAPILLARYS Hb A1c kit. The analyzed blood samples included 3 samples with normal Hba1c level (No. 1, 2 and 3), 1 sample with Hba1c level close to the cut-off value (No. 4) and 4 samples with elevated Hba1c level (No. 5, 6, 7 and 8). In this study, each blood sample was analyzed on the same capillary, including 40 runs over 20 working days (at 2 different times of the day). Within each run, samples were analyzed in duplicate. The results for Hba1c concentrations (in mmol/mol) and percentages are summarized in the following tables.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sample No. 1</th>
<th>Sample No. 2</th>
<th>Sample No. 3</th>
<th>Sample No. 4</th>
<th>Sample No. 5</th>
<th>Sample No. 6</th>
<th>Sample No. 7</th>
<th>Sample No. 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (Hba1c concentration – mmol/mol)</td>
<td>32</td>
<td>37</td>
<td>37</td>
<td>42</td>
<td>63</td>
<td>68</td>
<td>84</td>
<td>86</td>
</tr>
<tr>
<td>Within-run reproducibility (CV %)</td>
<td>2.3</td>
<td>1.7</td>
<td>2.2</td>
<td>1.1</td>
<td>1.4</td>
<td>1.5</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Between-run reproducibility (CV %)</td>
<td>2.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Between-day reproducibility (CV %)</td>
<td>0.0</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Total (CV %)</td>
<td>3.2</td>
<td>1.8</td>
<td>2.3</td>
<td>1.2</td>
<td>1.7</td>
<td>1.5</td>
<td>1.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Reproducibility between capillaries from the same instrument

Eight (8) different blood samples were run using the CAPILLARYS Hb A1c procedure in 8 capillaries of the same CAPILLARYS 2 FLEX-PIERCING instrument and with 1 lot of CAPILLARYS Hb A1c kit. The analyzed blood samples included 3 samples with normal Hba1c level (No. 1, 2 and 3), 1 sample with Hba1c level close to the cut-off value (No. 4) and 4 samples with elevated Hba1c level (No. 5, 6, 7 and 8). In this study, each blood sample was analyzed on all capillaries from the same instrument, including 40 runs over 20 working days (at 2 different times of the day). Within each run, samples were analyzed in duplicate. The results for Hba1c concentrations (in mmol/mol) and percentages are summarized in the following tables.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sample No. 1</th>
<th>Sample No. 2</th>
<th>Sample No. 3</th>
<th>Sample No. 4</th>
<th>Sample No. 5</th>
<th>Sample No. 6</th>
<th>Sample No. 7</th>
<th>Sample No. 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (Hba1c)</td>
<td>5.1</td>
<td>5.5</td>
<td>5.5</td>
<td>6.0</td>
<td>7.9</td>
<td>8.4</td>
<td>9.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Within-run reproducibility (CV %)</td>
<td>1.4</td>
<td>1.3</td>
<td>0.8</td>
<td>0.7</td>
<td>1.0</td>
<td>1.1</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Between-run reproducibility (CV %)</td>
<td>1.2</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Between-day reproducibility (CV %)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Total (CV %)</td>
<td>1.8</td>
<td>1.3</td>
<td>0.8</td>
<td>0.8</td>
<td>1.1</td>
<td>1.1</td>
<td>0.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Reproducibility between lots and instruments

Study No. 1

Eight (8) different blood samples were run using the CAPILLARYS Hb A1c procedure in 8 capillaries of 3 different CAPILLARYS 2 FLEX-PIERCING instruments and with 3 lots of CAPILLARYS Hb A1c kits. The analyzed blood samples included 3 samples with normal Hba1c level (No. 1, 2 and 3), 1 sample with Hba1c level close to the cut-off value (No. 4) and 4 samples with elevated Hba1c level (No. 5, 6, 7 and 8). In this study, each blood sample was analyzed on all capillaries from each instrument, including 60 runs over 24 working days (at 2 different times of the day). Within each run, samples were analyzed in duplicate. The following tables summarize the within-run and total instrument-reagent C.V. % ranges for the Hba1c concentrations (in mmol/mol) and percentages.
Four (4) different blood samples with elevated HbA1c level were run using the CAPILLARYS Hb A1c procedure in 8 capillaries of 2 CAPILLARYS 2 FLEX-PIERCING instruments and with 2 lots of CAPILLARYS Hb A1c kits. In this study, each blood sample was analyzed on all capillaries from each instrument, including 24 runs over 6 working days (at 2 different times of the day). Within each run, samples were analyzed in duplicate.

The following tables summarize the within-run and total instrument-reagent C.V. % ranges for the HbA1c concentrations (in mmol/mol) and percentages.

### Study No. 2 on blood samples with elevated HbA1c level

Four (4) different blood samples with elevated HbA1c level were run using the CAPILLARYS Hb A1c procedure in 8 capillaries of 2 CAPILLARYS 2 FLEX-PIERCING instruments and with 2 lots of CAPILLARYS Hb A1c kits. In this study, each blood sample was analyzed on all capillaries from each instrument, including 24 runs over 6 working days (at 2 different times of the day). Within each run, samples were analyzed in duplicate.

The following tables summarize the within-run and total instrument-reagent C.V. % ranges for the HbA1c concentrations (in mmol/mol) and percentages.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Mean (HbA1c concentration – mmol/mol)</th>
<th>Within-run reproducibility</th>
<th>Total reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV min (%)</td>
<td>CV max (%)</td>
<td>Total CV min (%)</td>
</tr>
<tr>
<td>Sample No. 1</td>
<td>31</td>
<td>2.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Sample No. 2</td>
<td>36</td>
<td>0.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Sample No. 3</td>
<td>37</td>
<td>1.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Sample No. 4</td>
<td>42</td>
<td>1.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Sample No. 5</td>
<td>63</td>
<td>0.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Sample No. 6</td>
<td>68</td>
<td>1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Sample No. 7</td>
<td>83</td>
<td>0.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Sample No. 8</td>
<td>86</td>
<td>0.8</td>
<td>1.9</td>
</tr>
<tr>
<td>CV (%) ranges</td>
<td></td>
<td>0.5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Mean (% HbA1c)</th>
<th>Within-run reproducibility</th>
<th>Total reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV min (%)</td>
<td>CV max (%)</td>
<td>Total CV min (%)</td>
</tr>
<tr>
<td>Sample No. 1</td>
<td>5.0</td>
<td>1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Sample No. 2</td>
<td>5.5</td>
<td>0.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Sample No. 3</td>
<td>5.5</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Sample No. 4</td>
<td>6.0</td>
<td>0.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Sample No. 5</td>
<td>7.9</td>
<td>0.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Sample No. 6</td>
<td>8.4</td>
<td>0.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Sample No. 7</td>
<td>9.8</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Sample No. 8</td>
<td>10.0</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>CV (%) ranges</td>
<td></td>
<td>0.4</td>
<td>2.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Mean (HbA1c concentration – mmol/mol)</th>
<th>Within-run reproducibility</th>
<th>Total reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV min (%)</td>
<td>CV max (%)</td>
<td>Total CV min (%)</td>
</tr>
<tr>
<td>Sample No. 1</td>
<td>97</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Sample No. 2</td>
<td>107</td>
<td>1.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Sample No. 3</td>
<td>119</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Sample No. 4</td>
<td>131</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>CV (%) ranges</td>
<td></td>
<td>1.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Mean (% HbA1c)</th>
<th>Within-run reproducibility</th>
<th>Total reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV min (%)</td>
<td>CV max (%)</td>
<td>Total CV min (%)</td>
</tr>
<tr>
<td>Sample No. 1</td>
<td>11.0</td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Sample No. 2</td>
<td>12.0</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Sample No. 3</td>
<td>13.0</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Sample No. 4</td>
<td>14.1</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CV (%) ranges</td>
<td></td>
<td>0.9</td>
<td>1.3</td>
</tr>
</tbody>
</table>

**Linearity**

The linearity of the CAPILLARYS Hb A1c procedure was evaluated in a study based on the Clinical Laboratory Standards Institute (CLSI - USA) EP6-A guideline "Evaluation of the Linearity of Quantitative Measurement Procedures: A statistical Approach".

The results for HbA1c concentration (mmol/mol) and percentage (%) were analyzed using statistical tools recommended by CLSI.

Two characteristic blood samples, including a normal sample with HbA1c concentration at 21 mmol/mol (4.0 % HbA1c) and an elevated HbA1c level sample with HbA1c concentration at 138 mmol/mol (14.7 % HbA1c) were mixed within different proportions and the dilutions were electrophoresed with the CAPILLARYS Hb A1c procedure. For each dilution, samples were analyzed in duplicate. The tests were determined to be linear within the entire ranges studied for HbA1c hemoglobin fraction.
In addition, 2 different characteristic blood samples, including a normal sample with HbA₁c concentration at 35 mmol/mol (5.4 % HbA₁c) and an elevated HbA₁c level sample with HbA₁c concentration at 61 mmol/mol (7.8 % HbA₁c), were both serially diluted in hemolysing solution and electrophoresed with the CAPILLARYS Hb A1c procedure. The tests were determined to be linear within the entire ranges studied from 1.4 to 31.0 g/dL total hemoglobin and HbA₁c fraction concentration and percentage were not affected by the hemoglobin concentration of the samples.

**Accuracy**

The following concordance study of the CAPILLARYS Hb A1c procedure performed with the CAPILLARYS 2 FLEX-PIERCING instrument was evaluated in a study based on the Clinical Laboratory Standards Institute (CLSI - USA) EP09-A2 guideline "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition (Interim Revision)".

The results for HbA₁c concentrations (mmol/mol) and percentages (%) were analyzed using statistical tools recommended by CLSI.

**NOTE:** The results presented below have been obtained from 1 external accuracy study that has been performed in 1 hospital laboratory located in the USA. The reference results were based on a routine HPLC procedure.

The levels of HbA₁c were measured in 282 blood samples, including 119 samples with normal HbA₁c levels and 163 samples with elevated HbA₁c levels, both by electrophoretic separations obtained with CAPILLARYS Hb A1c procedure with the CAPILLARYS 2 FLEX-PIERCING instrument and a commercially available HPLC instrument for HbA₁c quantification that is NGSP standardized.

The measured values of HbA₁c concentrations and percentages from both procedures were analyzed by a linear regression statistical procedure. The results of linear regression analysis are tabulated below (y = CAPILLARYS Hb A1c):

<table>
<thead>
<tr>
<th>HbA₁c</th>
<th>Correlation coefficient</th>
<th>y-Intercept</th>
<th>Slope</th>
<th>Range of values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (mmol/mol)</td>
<td>0.997</td>
<td>2.742</td>
<td>0.912</td>
<td>27 – 156</td>
</tr>
<tr>
<td>Percentage (%)</td>
<td>0.996</td>
<td>0.438</td>
<td>0.913</td>
<td>4.6 – 16.4</td>
</tr>
<tr>
<td>Page</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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PROFILS ÉLECTROPHORÉTIQUES - ELECTROPHORETIC PATTERNS

**Figure 1**

Profil normal  
*Normal pattern*

HbA1c : 5.1 % - 33 mmol/mol

**Figure 2**

Profil avec HbA1c augmentée  
*Pattern with elevated HbA1c level*

HbA1c : 8.3 % - 68 mmol/mol
Figure 3

Profil avec variant (Hb S suspectée)
*Pattern with variant (suspected Hb S)*

Figure 4

Profil avec variant (Hb C suspectée)
*Pattern with variant (suspected Hb C)*
Figure 5

Profil avec Hb F
Pattern with Hb F

PROFILS ÉLECTROPHORÉTIQUES - ELECTROPHORETIC PATTERNS