sebia

CAPI 3 Hb A1c

Ref. 2515

IVD

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INTENDED USE

The CAPI 3 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 3 instrument.
The CAPILLARYS 3 instrument is an automated analyzer which performs a complete hemoglobin profile for the quantitative analysis of HbA_{1c} fraction.
The hemoglobins, separated in silica capillaries, are directly detected by their absorbance at 415 nm.
The assay is performed on the hemolysate of whole blood samples collected in tubes containing K_2EDTA or K_3EDTA as anticoagulant.
Quantitative determination of hemoglobin A_{1c} is effective in monitoring middle-term blood glucose control in diabetic individuals.
Quantitative measurement of hemoglobin A_{1c} can be used as an aid in the diagnosis of diabetes mellitus and as an aid in the identification of patients at risk for developing diabetes mellitus.

The CAPI 3 Hb A1c procedure performed with the CAPILLARYS 3 instrument has been certified by the National Glycohemoglobin Standardization Program (NGSP).

For *In Vitro* Diagnostic Use.

NOTE : In this instruction sheet, the name "CAPILLARYS 3" is used for the SEBIA CAPILLARYS 3 OCTA, CAPILLARYS 3 TERA and CAPILLARYS 3 TERA TLA automated instruments.

PRINCIPLE OF THE TEST

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.
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,
- as an aid in the diagnosis of diabetes mellitus and as an aid in the identification of patients at risk for developing diabetes mellitus.

Electrophoresis is a well established technique routinely used in clinical laboratories for measuring components from body fluids, including HbA_{1c} glycated fraction. Besides the electrophoresis techniques performed on different media, including agarose gel and chromatography, the capillary electrophoresis has been developed to provide complete automation of this testing with fast separation and good resolution. It is defined as a technique of electrokinetic separation carried out in a tube of internal diameter lower than 100 μm filled with a buffer composed of electrolytes. In many aspects, the methodology can be considered as an intermediary type of technique between classical zone electrophoresis and liquid chromatography.
The CAPILLARYS 3 instrument uses the principle of capillary electrophoresis in free solution that is the most common form of capillary electrophoresis. 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 3 instrument has silica capillaries functioning in parallel allowing 8 simultaneous analyses (CAPILLARYS 3 OCTA) or 12 simultaneous analyses (CAPILLARYS 3 TERA and CAPILLARYS 3 TERA TLA) 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 CAPI 3 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 CAPI 3 Hb A1c KIT

WARNING : See the safety data sheets.

ITEMS	PN 2515
Buffer (ready to use)	2 vials, 700 mL each
Hemolysing solution (ready to use)	1 vial, 700 mL
Filters	4 filters

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

FOR OPTIMAL MANAGEMENT OF TRACEABILITY : All reagents from the same kit must be used together.
TO OBTAIN THE EXPECTED PERFORMANCES : The package insert instructions must be observed.

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 (between 2 and 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 (15 to 30 °C) ; 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 30 days, it may be stored at room temperature.
Once the buffer vial has been opened and positioned on the CAPILLARYS 3 instrument, it is stable for a maximum of **30 days** (accumulated) at room temperature (15 to 30 °C).

IMPORTANT : The accumulated time of the buffer stored at room temperature must not exceed **30 days**. This time of 30 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 (between 2 and 8 °C). It is stable until the expiration date indicated on the kit package or hemolysing solution vial label. DO NOT FREEZE.
Once the hemolysing solution vial has been opened and positioned on the CAPILLARYS 3 instrument, it is stable for a maximum of 2 months (accumulated). If the hemolysing solution vial is planned to be used for more than 2 months, it must be removed from the instrument after each use and stored at room temperature (15 to 30 °C) or refrigerated (between 2 and 8 °C), hemolysing solution is then stable until the expiration date indicated on the hemolysing solution vial label.
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. FILTERS

Use

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

IMPORTANT : When kit replacement, change systematically all the filters. Wear clean gloves for handling and installation of filters.

Screw one filter at the connector situated at the extremity of each tube that plunges in the vials of buffer, hemolysing solution and distilled or deionized water. When setting filters on the 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 (15 to 30 °C) or refrigerated (between 2 and 8 °C).

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 HbA_{1c} level and Hb A1c CAPILLARY Calibrator 2 presents an elevated HbA_{1c} level.

Intended use

The Hb A1c CAPILLARY Calibrators 1 and 2 are designed for the calibration and migration control of human glycated hemoglobin A_{1c} quantification with CAPI 3 Hb A1c electrophoresis procedure performed with the CAPILLARYS 3 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 3 instrument ;
  - 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 ;
  - in case of analyses of controls giving HbA_{1c} values outside the expected values (and after having confirmed this deviation by a second analysis of blood controls),
  - after having changed capillaries (whatever the number of replaced capillaries),
  - at least every 2 months.

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

Particular case : Calibration after having changed capillaries
- After having changed capillaries (whatever the number of replaced capillaries), perform 1 series of analyses with both calibrators, and then analyze both controls with the CAPILLARYS 3 sample rack No. 0.
- Check, for each analyzed control, that all obtained values fall within the specific range established for each lot of control, that are customized for the instrument (expected range previously established when setting the analyzed lots of controls).
- Check that the value deviation, obtained on each changed capillary is ≤ 0.2 point for control level 1 and ≤ 0.3 point for control level 2 compared to the mean value of the last 3 analyses of each control on each replaced capillary taken individually.

If the value deviation complies with expected specifications, the instrument can then be utilized for the analyses.
If not, perform 2 additional calibrations, and then analyze again both controls.
- Check, for each analyzed control, that all obtained values fall within the specific range established for each lot of control, that are customized for the instrument (expected range previously established when setting the analyzed lots of controls).
- Check that the value deviation, obtained on each changed capillary is ≤ 0.2 point for control level 1 and ≤ 0.3 point for control level 2 compared to the mean value of the last 3 analyses of each control on each replaced capillary taken individually.

If the value deviation complies with expected specifications, the instrument can then be utilized for the analyses.
If the value deviation does not comply with expected specifications (i.e., > 0.2 point for control level 1 and > 0.3 for control level 2), establish again customized values of controls according to the procedure indicated in the package insert of the Hb A1c CAPILLARY Controls.

IMPORTANT :
- For optimal use of each Hb A1c CAPILLARY Calibrator with the CAPILLARYS 3 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.
- Both calibrators must imperatively be analyzed for an effective calibration : the run order of both calibrators is indifferent, but they must be analyzed within the same working day.

- 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.
- Identify the tube with the specific bar code label of the calibrator.
- Place the tube with the calibrator in position No. 1 on the CAPILLARYS 3 sample rack No. 0.
- Slide the sample rack No. 0 into the CAPILLARYS 3 instrument, the analysis starts automatically.
- 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 : HbA_{1c} level in mmol/mol, lot number and expiration date, select the number of analyses of the calibrator to perform, according to the indications previously described, and validate.

  NOTE : HbA_{1c} concentration is indicated in IFCC unit (mmol/mol).

  WARNING : Do not enter the HbA_{1c} level in percentage.
- 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 HbA_{1c} value for both calibrators that has been entered by the operator.

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. MULTI-SYSTEM Hb A1c CAPILLARY CONTROLS (2)

Composition

Multi-System Hb A1c CAPILLARY Controls (SEBIA, PN 4768) 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 HbA_{1c} level and Hb A1c CAPILLARY Control 2 presents an elevated HbA_{1c} level.

Intended use

The Hb A1c CAPILLARY Controls 1 and 2 are designed for the migration control and quality control of human glycated hemoglobin A_{1c} quantification with CAPI 3 Hb A1c electrophoresis procedure performed with the CAPILLARYS 3 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 3 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 3 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 3 instrument,***
- ***after having changed one or many capillaries,***
- ***after having changed the lot number of calibrators or controls.***

See the package insert of the Multi-System Hb A1c CAPILLARY Controls.

Quality control :
It is recommended to analyze one of the two controls on whole capillaries as follows :
- after capillaries activation,
- after each calibration of the instrument performed with the Hb A1c CAPILLARY Calibrators,
- after a capillary cleaning sequence with CAPICLEAN.

In routine, it is recommended to analyze the Hb A1c control at the beginning and at the end of the analysis series, alternating the Control 1 and the Control 2.
In case of a series below 80 analyses : the control that will be analyzed at the beginning of the next series will allow to validate the results for samples from this series.
In the case of a migration shift leading to a non-recognition of fractions, it is recommended to analyze immediately one of the two Hb A1c controls.

- 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 Multi-System 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.
- Identify the tube with the specific bar code label of the control.
- Place the tube with the control in position No. 1 on the CAPILLARYS 3 sample rack No. 0.
- Start the analysis : Slide the sample rack into the CAPILLARYS 3 instrument.
- In the window which appears on the screen, select the number of analyses of the control to perform and validate.
- The results are then automatically considered by the software for the data analysis.
- Check the concentration levels and percentages for HbA_{1c} 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").

NOTES :

- *HbA_{1c} 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 HbA_{1c} level), statistics and Levey Jennings charts.*
- *It is recommended to use regularly the "Levey Jennings chart" function of the software to verify the absence of drift of the analysis of controls. In case of drift, perform a calibration of the instrument according to the procedure previously described.*

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 Multi-System Hb A1c CAPILLARY Controls.

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 for capillary electrophoresis CAPILLARYS 3, SEBIA.
It is recommended to use filtered distilled or deionized water (on a filter with a porosity ≤ 0.45 µm) and with a conductivity lower than 3 µS/cm, which corresponds to a resistivity higher than 0.33 MΩ.cm.
To prevent microbial proliferation, change the water every day.
For optimal operation, add CLEAN PROTECT (SEBIA, PN 2059, 1 vial of 5 mL) in distilled or deionized water (see the instructions for use of CLEAN PROTECT) or use directly the ready to use CAPIprotect* solution (SEBIA, PN 2061 : 2 containers of 5 L of distilled water with CLEAN PROTECT).

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

** NOTE : The CAPIprotect solution can also be used to dilute the stock wash solution. Then, in that case, the diluted wash solution may show a transient more or less marked yellow colour without any adverse effects on its performance.*

4. CAPILLARYS 3 CAPICLEAN

Composition

The vial of CAPICLEAN concentrated solution (SEBIA, PN 2060, 1 vial of 25 mL) contains : proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, necessary for optimum performances.

Use

For sample probe cleaning in automated instrument for capillary electrophoresis CAPILLARYS 3, SEBIA, during the CAPICLEAN cleaning sequence.

IMPORTANT :

- When less than 500 samples are analyzed within a week, launch a CAPICLEAN cleaning sequence at least once a week.
- When less than 500 samples are analyzed within a day but more than 500 samples are analyzed within a week, launch a CAPICLEAN cleaning sequence after every 500 analyses.
- When more than 500 samples are analyzed within a day, launch a CAPICLEAN cleaning sequence once a day.

See the instruction sheets of CAPILLARYS 3 CAPICLEAN and the instruction manual of CAPILLARYS 3, SEBIA.

Storage, stability and signs of deterioration

Store CAPICLEAN refrigerated (between 2 and 8 °C). It is stable until the expiration date indicated on the vial label. DO NOT FREEZE.
Precipitate or combined particles in suspension (floccules) may be observed in the CAPICLEAN vial without any adverse effects on its utilization.
Do not to 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 3 Instrument, SEBIA (weekly maintenance in order to eliminate adsorbed proteins from the probe).

See the CAPILLARYS 3 instruction manual, SEBIA.

Storage, stability and signs of deterioration

Store the working chlorinated solution at room temperature (15 to 30 °C) 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 3 WASH SOLUTION

Preparation

The vial of the stock wash solution (SEBIA, PN 2062, 1 vial, 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 after electrophoretic separation.

IMPORTANT :

- When wash solution vial replacement, change systematically the filter. Wear clean gloves for handling and installation of the filter.
- Before placing the wash solution vial in the instrument, it is recommended to wash the connector and the tube with plenty of distilled or deionized water to avoid salts deposit.
- Screw the filter at the connector situated at the extremity of the tube plunging in the wash solution vial.

Storage, stability and signs of deterioration

Store the stock and working wash solutions in closed containers at room temperature (15 to 30 °C) or refrigerated (between 2 and 8 °C).
The stock wash solution is stable until the expiration date indicated on the kit or wash solution vial label.
Working wash solution is stable for 3 months.
After dilution and immediate installation of the vial in the instrument, the solution is stable for 3 months (if the working wash solution is stored out of the instrument before use, this time of 3 month storage must take into account the time during which the solution is stored outside the instrument).
Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

7. SALINE

Preparation

Make 0.15 M (0.9 g/dL) NaCl solution in distilled or deionized water.

Use

To wash red blood cells from samples (see § Sample preparation, Particular cases).

Storage, stability and signs of deterioration

Store saline at room temperature (15 to 30 °C) or refrigerated (2 - 8 °C).
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.

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 filter ≤ 0.45 µm) and have a conductivity lower than 3 µS/cm, which corresponds to a resistivity higher than 0.33 MΩ.cm.

EQUIPMENT AND ACCESSORIES REQUIRED

1. SEBIA CAPILLARYS 3 instrument for capillary electrophoresis : CAPILLARYS 3 OCTA PN 1245, CAPILLARYS 3 TERA PN 1246 or CAPILLARYS 3 TERA TLA PN 1248, connected to a computer equipped with the PHORESIS software for data processing.
2. Sample racks supplied with CAPILLARYS 3 instrument.
3. CAPILLARYS 3 & MC SWITCH RACK FOR HbA1c (1), SEBIA, PN 1383, to launch automatically a technique change to HbA1c procedure on the CAPILLARYS 3 instrument.
4. CAPILLARYS 3 & MC CAPILLARY BLOOD RACKS (5), SEBIA, PN 1363, for the analysis of samples with a volume comprised between 20 and 100 µL on the CAPILLARYS 3 instrument.
5. CAPILLARYS 3 & MC LOW VOLUMES RACKS (5), SEBIA, PN 1364, for the analysis of samples with a volume comprised between 100 and 400 µL on the CAPILLARYS 3 instrument.
6. Container Kit supplied with CAPILLARYS 3 instrument : Rinse vial (to fill with distilled or deionized water), wash solution vial, waste container and external waste container (for CAPILLARYS 3 TERA TLA).
7. CAPI 3 REAGENT CUPS (24 x 14), SEBIA, PN 2582, including 24 packs of 14 CAPI 3 reagent cups : Single use cups for the preparation of biological samples to analyze with the automated instrument. To be placed on the automated loading system for cups of CAPILLARYS 3. One reagent cup is intended for the analysis of 8 samples with CAPILLARYS 3 OCTA and 12 samples with CAPILLARYS 3 TERA and CAPILLARYS 3 TERA TLA.

   WARNING : After use, reagent cups with biological samples have to be handled with care. When the analysis is completed, reagent cups must be discarded with biological waste products and they must NEVER be reused.

   Storage : Before use, store the reagent cups in their sealed package in a clean and dry place and at a temperature comprised between 2 and 30 °C.
8. CAPI 3 BINS FOR USED REAGENT CUPS (5), SEBIA, PN 2581 : Bins intended for automated collection of used reagent cups in CAPILLARYS 3 OCTA and CAPILLARYS 3 TERA. To place at the location intended for this purpose.

   WARNING : Bins containing used reagent cups with biological samples have to be handled with care.
9. Container for recovery of biological waste (not marketed by SEBIA, maximal capacity of 12 litres) : Container intended for automated collection of used reagent cups in CAPILLARYS 3 TERA TLA. To place at the location intended for this purpose.
10. Collection tubes with 13 mm diameter and their corresponding caps (maximal length of tube with cap : 91 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 2.6, 2.7 or 3.4 mL tubes (13 x 75 mm),
    or
    collection tubes with 11 mm diameter and their corresponding caps (maximal length of tube with cap : 91 mm, maximal diameter of cap : 17 mm) : for example, Sarstedt S-Monovette 2.7 mL or Kabe Labortechnik Primavette S 2.6 mL tubes (11 x 66 mm),
    or collection tubes with equivalent dimensions approved for clinical assays.

    WARNING : Do not use these collection tubes on a sample rack No. 0 from the CAPILLARYS 3 instrument (the sample rack No. 0 must only be used with conical tubes for the analysis of calibrators and blood controls).
11. Tubes and caps for Controls, SEBIA, PN 9202 (20 units) or PN 9205 (500 units) : conical tubes and their caps to analyze calibrators, blood controls and samples with a low volume (see § Sample preparation), with the CAPILLARYS 3 instrument.
12. A1c/CE LOW VOLUME SAMPLE COLLECTION TUBE, SEBIA, PN 9216 (200 tubes), for the analysis of samples with a volume below 100 µL on the CAPILLARYS 3 instrument.
13. TEST TUBES, SEBIA, PN 9214 : 200 100 mm-tubes for the hypochlorite sodium solution intended for the cleaning of the sample probe, or tubes (without cap) with equivalent dimensions (length comprised between 90 and 100 mm and diameter comprised between 13 and 16 mm).

SAMPLES FOR ANALYSIS

Sample collection and storage

Fresh anticoagulated whole blood samples collected in tubes containing K_2EDTA or K_3EDTA 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 72 hours maximum at room temperature (between 15 and 30 °C).
For longer storage, samples can be frozen at - 70 / - 80 °C within 8 hours of collection without any preparation.
Frozen blood samples are stable for 3 months maximum at - 70 / - 80 °C.

IMPORTANT : For optimal storage of blood samples, store them at - 70 / - 80 °C. Do not store at - 20 °C.

Sample storage may induce hemoglobin degradation and then, the electrophoretic pattern may be affected by degradation products (as artefacts). When analyzing such samples, an additional fraction may migrate particularly :
- more cathodically than Hb A2 and / or,
- more cathodically than Hb A0 (between Hb A0 and Hb A2) and / or,
- more anodically than Hb A0 (in the "other Hb A" migration position).

In such cases, the HbA_{1c} value is not affected.

Sample preparation

- Use directly whole blood samples.
- Check that all the tubes contain 800 µL minimum of blood and are perfectly closed.
- **Vortex for 5 seconds blood samples stored at 2 – 8 °C for one week or stored at - 70 / - 80 °C.**

WARNING : The tubes must be closed with their corresponding caps designed for the CAPI 3 Hb A1c procedure with the CAPILLARYS 3 instrument (see EQUIPMENT AND ACCESSORIES REQUIRED).

Particular cases :
Analysis of samples with a low volume :
The following table presents the tubes and sample racks to use according to the minimum volume of sample to analyze.

	STANDARD TUBE	STANDARD TUBE (EXCEPT FOR Sarstedt S-Monovette tubes)	TUBES AND CAPS FOR CONTROLS (conical tubes, PN 9202 & 9205)		A1c/CE LOW VOLUME SAMPLE COLLECTION TUBE (PN 9216)
	CAPILLARYS 3 & MC SAMPLE RACKS (PN 1369)	CAPILLARYS 3 & MC LOW VOLUMES RACKS (PN 1364)	CAPILLARYS 3 & MC SAMPLE RACKS (PN 1369)	CAPILLARYS 3 & MC LOW VOLUMES RACKS (PN 1364)	CAPILLARYS 3 & MC CAPILLARY BLOOD RACKS (PN 1363)
Minimum volume of sample needed for the CAPI 3 Hb A1c analysis	800 µL	300 µL (1)	400 µL (2)	100 µL (3)	20 µL (4)
Software version for Hb A1c	all	≥ 1.06	all	≥ 1.06	≥ 1.06
Handling	No handling of the sample --> complete traceability	No handling of the sample --> complete traceability	Apply a minimum of 400 µL of sample in a conical tube	Apply a minimum of 100 µL of sample in a conical tube	Apply 20 µL of sample in a transport tube

(1) Analysis of samples with a volume comprised between 300 and 800 µL (EXCEPT for Sarstedt S-Monovette tubes) :
- Place the capped tube with whole blood sample to analyze (at least 300 µL) on a CAPILLARYS 3 & MC LOW VOLUMES rack.
- Slide the rack into the CAPILLARYS 3 instrument.

WARNING : Do not use Sarstedt S-Monovette collection tubes on a CAPILLARYS 3 LOW VOLUMES rack (important risk of instrument and tube damage). For samples with a volume below 800 µL in this type of tube, follow the procedure that corresponds to the volume to analyze.

(2) Analysis of samples with a volume comprised between 400 and 800 µL :
- Vortex for 5 seconds the whole blood sample to analyze.
- Apply in a conical tube for control the whole blood sample (at least 400 µL) and cap the tube.
- Identify the tube with the specific bar code label of the sample.
- Place the tube on a CAPILLARYS 3 & MC SAMPLE rack.
- Slide the rack into the CAPILLARYS 3 instrument at the beginning of an analysis series.

NOTE : It is recommended to gather samples with a volume comprised between 400 and 800 µL 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.

(3) Analysis of samples with a volume comprised between 100 and 300 µL :
- Vortex for 5 seconds the whole blood sample to analyze.
- Apply in a conical tube for control the whole blood sample (at least 100 µL) and cap the tube.
- Identify the tube with the specific bar code label of the sample.
- Place the tube on a CAPILLARYS 3 & MC LOW VOLUMES rack.
- Slide the rack into the CAPILLARYS 3 instrument at the beginning of an analysis series.

NOTE : It is recommended to gather samples with a volume comprised between 100 and 300 µL 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 CAPILLARYS 3 & MC LOW VOLUMES rack into the automated instrument. Without any bar code label on the conical tube, the sample cannot be identified.

(4) Analysis of samples with a volume comprised between 20 and 100 µL :
- Vortex for 5 seconds the whole blood sample to analyze.
- Apply 20 µL of whole blood sample in a tube "A1C/CE LOW VOLUME SAMPLE COLLECTION TUBE" and cap the tube.
- Vortex the tube for 5 seconds.
- Identify the tube with the specific bar code label of the sample.
- Place the tube on a CAPILLARYS 3 & MC CAPILLARY BLOOD rack.
- Slide the rack into the CAPILLARYS 3 instrument.
- When the analysis is completed, discard the tube with biological waste (a second analysis of the sample cannot be performed with the remaining sample).

Analysis of red blood cells :
Prepare red blood cells according to the following procedure :
- Centrifuge the whole blood to obtain a red blood cells pellet.
- Remove the plasma and measure the volume of plasma removed.
- Wash the red blood cells 3 times with 10 volumes of saline (centrifuge after each washing step).
- Discard the excess of saline over the red blood cells pellet.
- Apply the red blood cells in a conical tube for control.
- Mix the red blood cells with a volume of saline equal to the volume of removed plasma (minimal final volume of sample = 1 mL).

- Cap the tube.
- Vortex for 5 seconds.
- Perform the analysis of this sample according to the standard procedure.

NOTE : Identify the conical tube. 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 that affect the collection of the sample by the probe. Important hemoglobin degradation products (as artefacts) may also affect the electrophoretic pattern and additional fractions may migrate particularly more cathodically than Hb A2 and / or more cathodically than Hb A0 (between Hb A0 and Hb A2) and / or more anodically than Hb A0 (in the "other Hb A" migration position).
- Do not analyze directly tubes containing less than 800 µL of blood sample, the analysis should be affected.

PROCEDURE

The CAPILLARYS 3 instrument is a multiparameter instrument for hemoglobins analysis on parallel capillaries. The hemoglobins assay uses 8 or 12 capillaries to run the samples.
The sequence of automated steps is as follows :

- sample racks identification by RFID (Radio Frequency Identification),
- bar code reading of sample tubes (for up to 8 tubes),
- mixing of blood samples before analysis,
- sample hemolysis and dilution from primary tubes into reagent cups,
- 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,
- placement of racks on the CAPILLARYS 3 instrument,
- removal of sample racks and sample tubes after analysis,
- removal of bins for used reagent cups.

PLEASE CAREFULLY READ THE CAPILLARYS 3 INSTRUCTION MANUAL.

I. PREPARATION OF CAPILLARYS ANALYSIS

1. Switch on CAPILLARYS 3 instrument and computer.
2. Wait until the instrument is completely initialized.
3. Start the PHORESIS software installed on the computer for data processing.
4. The CAPI 3 Hb A1c kit is intended to run with "HbA1c" analysis program from the CAPILLARYS 3 instrument. To select "HbA1c" analysis program and place the CAPILLARYS Hb A1c buffer and hemolyzing solution vials in the instrument, please read carefully the CAPILLARYS 3 instruction manual. If necessary, place the vial with the reconstituted wash solution in the instrument.
5. The sample rack contains 8 positions for sample tubes. Place up to 8 capped sample tubes with whole blood on each sample rack ; the bar code of each tube must be visible in the openings of the sample rack.
6. Take a pack of new reagent cups by holding the handle and place it on the automated loading system for cups of CAPILLARYS 3 ; then, remove the flange (a message will be displayed when reagent cups are missing).
7. Place a new bin for used reagent cups into the CAPILLARYS 3 instrument at the location intended for this purpose.
8. Slide the sample rack(s) into the CAPILLARYS 3 instrument through the opening in the right side of the instrument. Up to 15 sample racks can be introduced successively and continuously into the instrument.

   NOTES :

   - *When analyzing a control blood sample, it is advised to use specific conical tubes for control bloods and their corresponding caps, and a rack No. 0 for controls or a sample rack.*
   - *Do not analyze blood samples on a sample rack No. 0, the analysis should be affected.*
9. Remove analyzed sample racks from the plate on the left side of the instrument.
10. If necessary, take off carefully the bin containing used reagent cups and discard it.

    WARNING : Bins containing used reagent cups with biological samples have to be handled with care.

DILUTION - MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

1. Sample rack identification by RFID.
2. Bar codes are read on primary sample tubes.
3. Mixing of tubes.
4. Samples are diluted in hemolysing solution and the sample probe is rinsed after each sample.
5. Capillaries are washed.
6. Diluted samples are injected into capillaries.
7. Migration is carried out under constant voltage for about 9 minutes and the temperature is controlled by Peltier effect.
8. Hemoglobins are detected directly by scanning at 415 nm and data of the obtained hemoglobin electrophoretic pattern are transmitted from the instrument to the computer equipped with the software for data processing.

NOTE : Please read the CAPILLARYS 3 TERA TLA instruction manual for the analysis of tubes delivered by a laboratory automation system.

II. RESULT ANALYSIS

At the end of each analysis, the corresponding data are transmitted by the instrument to the software for data processing and a hemoglobin electrophoretic profile appears on the screen of the computer. Relative quantification of individual HbA_{1c} fraction is performed automatically.
The HbA_{1c} concentrations are standardized and indicated in percentages (with one decimal place) and in mmol/mol (without any decimal place) according to IFCC recommendations (Ragnar Hanas *et al*, 2010).

NOTE : As the sample is characterized as normal or with elevated HbA_{1c} level using the real value of HbA_{1c} concentration in mmol/mol (whole number calculated by the software for the data analysis), a discordance may appear for HbA_{1c} levels close to the threshold value.

The identification of normal blood samples and of blood samples with elevated HbA_{1c} 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 HbA_{1c} level :
- Normal blood samples, with "normal" HbA_{1c} concentration lower than 42 mmol/mol (6.0 %) or equal are indicated in blue color.
- Blood samples with elevated HbA_{1c} 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 HbA_{1c}, 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>
<tr><th>Warning signal
Analyzed sample</th><th>HbA_{1c} value outside specifications for calibrators</th><th>No detection of Hb A0 and / or HbA_{1c} fraction</th><th>Insufficient optical density for Hb A0 fraction</th><th>"Atypical profile" (presence of an additional fraction or deletion of a normal fraction)</th><th>HbA_{1c} value outside the expected values for controls analyzed with the Quality Control (QC) mode</th></tr>
<tr><td>Calibrators identified with bar code labels</td><td colspan="4">Warning message: "analysis of the calibrator not in conformity": analyze a control blood with a sample rack No. 0 or repeat the calibration [in case of invalid calibration on one (or many) capillary(ies), the capillary(ies) is(are) deactivated, the HbA_{1c} level is then not displayed].</td><td>/</td></tr>
<tr><td rowspan="2">Controls identified with bar code labels</td><td>/</td><td>No HbA_{1c} value displayed</td><td>/</td><td>/</td><td>With the Quality Control mode: "+" or "-" identification according to the HbA_{1c} level compared to the customized values entered by the operator.</td></tr>
<tr><td>/</td><td colspan="3">Warning message: "analysis of the control not in conformity":
1. repeat the analysis with a sample rack No. 0 using the same vial,
2. repeat the analysis with a new vial,
3. call SEBIA Technical Service if the failure is confirmed.</td><td>With the Quality Control mode, if the warning message "analysis of the control not in conformity" is displayed, repeat the calibration.</td></tr>
<tr><td>Blood sample from patient</td><td>No HbA_{1c} value displayed (when shoulder on HbA_{1c} and / or on Hb A0)</td><td>Suspect the presence of a Hb variant or a failure in the adjustment of the electrophoretic patterns, repeat the analysis for confirmation.</td><td>Warning message: "too low OD" (< 0.06) for a blood sample that is not abnormal, repeat the analysis: if the result is confirmed, the HbA_{1c} level can be reported.</td><td>"atypical profile" and "HbA_{1c} (*)" : suspect the presence of a Hb variant.</td><td>/</td></tr>
</table>

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 HbA_{1c} synthesis, may be suspected (case of physio-pathological interference). It is recommended to analyze the sample with the HEMOGLOBIN(E) procedure to verify the Hb A2 percentage and to study the patient's clinical data. However, the HbA_{1c} quantification represents a useful relative follow up index for the same patient.

PLEASE CAREFULLY READ THE PHORESIS 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 3 instrument in order to store capillaries in optimal conditions.

IV. FILLING OF REAGENT CONTAINERS AND MANAGEMENT OF DISPOSABLES

The CAPILLARYS 3 instrument has an automatic control for reagents (by using RFID labels) and for disposables (reagent cups and bins for used cups).

IMPORTANT : It is necessary to respect the designed position for wash solution, rinse and waste containers.

On the screen of the CAPILLARYS 3 instrument, the "Main compartment" menu for reagents management displays information when it is necessary to perform one of the following tasks :
- place a new buffer vial 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 3 INSTRUCTION MANUAL.

QUALITY CONTROL

See "REAGENTS REQUIRED BUT NOT SUPPLIED", § "Hb A1c CAPILLARY CONTROLS".

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 3 instrument, it is necessary to use the specific conical tubes for controls and their corresponding caps (see "EQUIPMENT AND ACCESSORIES REQUIRED") and the bar code labels intended to identify the tubes for controls that contain the blood control to analyze.

RESULTS

Values

Direct detection at 415 nm in capillaries yields relative concentrations (percentages) of individual hemoglobin zones, and specially the calibrated HbA_{1c} concentration.

HbA_{1c} threshold value for diagnosis of diabetes mellitus :
Refer to the current local specific guidelines for the cutoff of HbA_{1c} for the diagnosis of diabetes mellitus.

Hemoglobin A_{1c} expected value range was cited from the American Diabetes Association (Standards of Medical Care in Diabetes – 2017. Diabetes Care 2017, 40 (Suppl. 1)).
The American Diabetes Association's (ADA) most recent Clinical Practice are :

Category	HbA_{1c} Range (IFCC)	HbA_{1c} Range (NGSP/DCCT)
Normal	< 39 mmol/mol	< 5.7 %
Prediabetes (increased risk for diabetes)	39 mmol/mol - 47 mmol/mol	5.7 % - 6.4 %
Diabetes	≥ 48 mmol/mol	≥ 6.5 %

The expected HbA_{1c} range for non-diabetic adults is 20 – 42 mmol/mol or 4.0 – 6.0 %. However, each laboratory should check (or establish) the reference range and HbA_{1c} goal in their country of business taking into account sex, age, ethnicity and individual patient situation.

WARNING : The HbA_{1c} value obtained for a sample that presents a hemoglobin variant cannot be compared to the HbA_{1c} normal value to make a diagnosis. Actually, according to IFCC and NGSP recommendations, the normal value has been established for individuals without any hemoglobinopathy. For an atypical sample (with a variant), this HbA_{1c} normal value is not displayed by the software but HbA_{1c} 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 HbA_{1c} quantitative determination were evaluated in studies based on the Clinical Laboratory Standards Institute (CLSI - USA) EP7-A2 guideline "Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition".

The results are summarized below :

- No interference with the CAPI 3 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 :

Endogenous interfering factor	**Concentration**
Bilirubin	70 mg/dL (1197 µM)
D-glucose	1000 mg/dL (55 mM)
Rheumatoid factor	1294 IU/mL
Total Protein	149.5 g/L
Triglycerides	3.58 g/dL (40.9 mM)
Urea	265 mg/dL (44.2 mM)

Drug	**Concentration**
Acetaminophen	200 mg/L (1325 µM)
Acetylcysteine	200 mg/dL (12.3 mM)
Acetylsalicilyc acid	1000 mg/dL (55.56 mM)
Ampicillin-Na	1000 mg/dL (28653 µM)
Ascorbic acid	300 mg/dL (17045 µM)
Cefoxitin	2500 mg/dL (58548 µM)
Cyclosporine	5 mg/L
Doxycycline	50 mg/dL (1123.6 µM)
Glybenclamide	3 mg/dL
Heparin	5000 U/L
Ibuprofen	500 mg/L (2427 µM)
Levodopa	40 mg/dL
Metformin	5 mg/dL
Methyldopa	40 mg/dL (1896 µM)
Metronidazole	200 mg/dL (11696 µM)
Phenylbutazone	400 mg/L
Rifampicin	70 mg/L (85.1 µM)
Theophylline	100 mg/L (556 µM)

Hemoglobin derivatives and cross reactants:

- No interference with the CAPI 3 Hb A1c procedure was detected due to the presence of carbamylated hemoglobin (≤ 8.1 mg/mL or 7.2 %), HbA1a+b (≤ 0.20 mg/mL) and labile HbA_{1c} (≤ 20.0 mg/mL or 20.8 %).
- Acetylated hemoglobin may migrate in minor hemoglobins migration zone, no interference has been observed with HbA_{1c} fraction quantification due to the presence of acetylated hemoglobin (≤ 4.2 mg/mL or 3.6 %).
- No interference with the CAPI 3 Hb A1c procedure was detected due to the presence of glycated albumin (≤ 2.2 mg/mL).

Analysis with hemoglobin variants:

- Levels of Hb F up to 23 % in the blood sample do not interfere with HbA_{1c} fraction quantification, a result is reported by the software when the Hb F level is higher than 23 % along with a warning message "Atypical profile – Possible quantitative interference if Hb F or variant > 23 %".
- Levels of Hb A2 up to 7.8 % in the blood sample do not interfere with HbA_{1c} fraction quantification.
- No interference has been observed with HbA_{1c} fraction quantification due to the presence of major abnormal hemoglobins Hb S (≤ 40.8 %), Hb C (≤ 37.6 %), Hb D (≤ 41.3 %) and Hb E (≤ 26.8 %). However, due to the number of variants, the presence of another hemoglobin variant may be observed in the HbA_{1c} migration zone; in the case of a shoulder on HbA_{1c}, no result will be reported by the software (as in presence of Hb Bart's).
- 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 HbA_{1c} 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. 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 HbA_{1c}. The electrophoretic pattern will be identified as "Atypical profile". Do not report any HbA_{1c} 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 HbA_{1c} result will be reported by the software due to the absence of HbA_{1c} in this kind of sample.
- Some hemoglobin variants (for example J Baltimore) can migrate close enough to the HbA_{1c} fraction to disturb the quantification (underestimation due to insufficient return to baseline). If a result is reported, this result will be labeled as "Atypical Profile" and should be reviewed by the operator.

- Individuals with recent significant blood loss exhibit falsely low HbA_{1c} values due to a higher fraction of young erythrocytes.
- Abnormal life span of red blood cells, as found in hemolytic anemias, polycythemia or postsplenectomy, may affect the levels of HbA_{1c}. However, the values represent a useful relative follow up index for the same patient.
- For some patients, the migration speed of the samples may be accelerated causing a shift of the profile that may result in a non-recognition of the fractions (this may be observed in some cases of patients with hyperleukocytosis for example). It is then recommended to wash the red blood cells and to re-analyze the samples according to the standard procedure (see § Sample preparation, Particular cases).

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 800 µL of blood sample.
- Avoid aged, improperly stored blood samples ; important degradation products (or artefacts) may affect the electrophoretic pattern after 7 days storage. When analyzing such samples, additional fractions may migrate particularly more cathodically than Hb A2 and / or more cathodically than Hb A0 (between Hb A0 and Hb A2) and / or more anodically than Hb A0 (in the "other Hb A" migration 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 HbA_{1c} may not be quantified in presence of all hemoglobin variants with this method.
- The CAPI 3 Hb A1c kit should not be used:
  - for "Point-of-Care" use,
  - in monitoring daily glucose control,
  - to replace daily home testing of urine and blood glucose levels,
  - to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes,
  - for analyzing samples from patients with total hemoglobin levels of less than 2.9 or greater than 30.5 g/dL and any hemoglobinopathies that may interfere,
  - to diagnose diabetes during pregnancy or to diagnose gestational diabetes. HbA_{1c} reflects the average blood glucose levels over the preceding 3 months (the average life of a red blood cell), and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red cell survival,
  - to diagnose diabetes in patients with the following conditions:
    - Any condition that alters the life span of the red blood cells, including recent blood loss, transfusion, significant iron deficiency, hemolytic anemia (including hereditary spherocytosis) or other hemolytic diseases, hemoglobinopathies and thalassemias, as the altered red blood cell turnover interferes with the relationship between mean blood glucose and HbA_{1c} values,
    - Malignancies or severe chronic hepatic and renal disease.
- In cases of rapidly evolving Type 1 diabetes, the increase of HbA_{1c} values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.
- A significant negative interference has been observed with fetal hemoglobin (Hb F) concentrations > 23 %. HbA_{1c} results are invalid for patients with high amounts of Hb F (> 23 %) including those with known Hereditary Persistence of Fetal Hemoglobin.

Hemoglobin variants observed with Hb A1c and / or HEMOGLOBIN(E) procedures :
Due to the different composition of Hb A1c and HEMOGLOBIN(E) buffers, the electrophoretic mobility of some hemoglobin variants may be different.

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 SEBIA's extranet website : www.sebia.com.

PERFORMANCE DATA

Precision

The precision of the CAPI 3 Hb A1c procedure was evaluated in a study based on the Clinical Laboratory Standards Institute (CLSI - USA) EP5-A3 guideline "Evaluation of Precision of Quantitative Measurements Procedures; Approved Guideline – Third Edition".
The means, standard deviations (SD) and coefficients of variation (CV %) were calculated for HbA_{1c} concentration (mmol/mol) and percentage (%) for each sample.

Eight (8) different blood samples were run using the CAPI 3 Hb A1c procedure on 3 CAPILLARYS 3 instruments. The analyzed blood samples included 3 samples with normal HbA_{1c} level (No. 1, 2 and 3), 1 sample with HbA_{1c} level close to the cut-off value (No. 4) and 4 samples with elevated HbA_{1c} level (No. 5, 6, 7 and 8).
Each sample was analyzed in duplicate on two capillaries per run, two runs per day over 24 days per lot of CAPI 3 Hb A1c kit, using three lots yielding a total of 1728 results per sample over 72 days.

The reproducibility between instruments is summarized in the following tables including within-capillary (repeatability), between-capillary, between-run, between-day, between-lot, between-instrument and total reproducibility precision estimates (SD and %CV) for the HbA_{1c} concentrations (in mmol/mol) and percentages.

①	Mean (mmol/mol)	Within-capillary		Between-capillary		Between-run		Between-day		Between-lot		Between-instrument		Total reproducibility (*)	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	33	0.50	1.5%	0.48	1.4%	0.00	0.0%	0.24	0.7%	0.40	1.2%	0.18	0.6%	0.86	2.6%
Blood No. 2	34	0.49	1.4%	0.52	1.5%	0.00	0.0%	0.28	0.8%	0.49	1.5%	0.00	0.0%	0.91	2.7%
Blood No. 3	37	0.44	1.2%	0.51	1.4%	0.00	0.0%	0.27	0.7%	0.42	1.1%	0.00	0.0%	0.84	2.3%
Blood No. 4	48	0.56	1.2%	0.43	0.9%	0.26	0.5%	0.22	0.5%	0.38	0.8%	0.00	0.0%	0.83	1.7%
Blood No. 5	61	0.53	0.9%	0.43	0.7%	0.00	0.0%	0.43	0.7%	0.09	0.1%	1.52	2.6%	1.72	2.9%
Blood No. 6	65	0.62	1.0%	0.43	0.7%	0.00	0.0%	0.31	0.5%	0.23	0.4%	0.13	0.2%	0.86	1.3%
Blood No. 7	86	0.64	0.7%	0.49	0.6%	0.00	0.0%	0.39	0.5%	0.72	0.8%	0.00	0.0%	1.14	1.3%
Blood No. 8	107	0.74	0.7%	0.83	0.8%	0.00	0.0%	0.67	0.6%	1.79	1.7%	0.00	0.0%	2.21	2.1%

(*) Total reproducibility includes : within-capillary, between-capillary, between-run, between-day, between-lot and between-instrument.

②	Mean (%)	Within-capillary		Between-capillary		Between-run		Between-day		Between-lot		Between-instrument		Total reproducibility (*)	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	5.2	0.05	0.9%	0.04	0.8%	0.00	0.0%	0.02	0.4%	0.04	0.7%	0.02	0.3%	0.08	1.5%
Blood No. 2	5.2	0.05	0.9%	0.05	0.9%	0.00	0.0%	0.03	0.6%	0.04	0.7%	0.00	0.0%	0.08	1.6%
Blood No. 3	5.5	0.04	0.8%	0.04	0.8%	0.00	0.0%	0.03	0.5%	0.04	0.7%	0.00	0.0%	0.08	1.4%
Blood No. 4	6.5	0.05	0.7%	0.04	0.5%	0.02	0.3%	0.02	0.3%	0.03	0.5%	0.00	0.0%	0.07	1.1%
Blood No. 5	7.7	0.05	0.7%	0.04	0.5%	0.00	0.0%	0.04	0.5%	0.01	0.2%	0.13	1.7%	0.15	2.0%
Blood No. 6	8.1	0.06	0.7%	0.04	0.5%	0.00	0.0%	0.03	0.4%	0.02	0.3%	0.01	0.1%	0.08	1.0%
Blood No. 7	10.1	0.06	0.6%	0.05	0.5%	0.00	0.0%	0.04	0.4%	0.07	0.7%	0.00	0.0%	0.11	1.1%
Blood No. 8	11.9	0.07	0.6%	0.08	0.6%	0.00	0.0%	0.06	0.5%	0.16	1.4%	0.00	0.0%	0.20	1.7%

(*) Total reproducibility includes : within-capillary, between-capillary, between-run, between-day, between-lot and between-instrument.

The reproducibility within the same instrument is summarized in the following tables:
- including within-capillary (repeatability), between-capillary, between-run, between-day, between-lot and total reproducibility precision estimates (SD and %CV) for the HbA_{1c} concentrations (in mmol/mol) and percentages for each instrument.
- including the within-lot precision estimates (SD and %CV) for the HbA_{1c} concentrations (in mmol/mol) and percentages for each lot on each instrument.

Instrument No. 1

③	Mean (mmol/mol)	Within-capillary		Between-capillary		Between-run		Between-day		Between-lot		Total reproducibility (*)	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	33	0.50	1.5%	0.42	1.3%	0.00	0.0%	0.19	0.6%	0.42	1.3%	0.80	2.4%
Blood No. 2	34	0.42	1.3%	0.49	1.4%	0.00	0.0%	0.28	0.8%	0.62	1.8%	0.94	2.8%
Blood No. 3	37	0.47	1.3%	0.44	1.2%	0.00	0.0%	0.30	0.8%	0.45	1.2%	0.84	2.3%
Blood No. 4	48	0.55	1.1%	0.38	0.8%	0.18	0.4%	0.28	0.6%	0.49	1.0%	0.89	1.9%
Blood No. 5	61	0.51	0.9%	0.50	0.8%	0.00	0.0%	0.37	0.6%	0.07	0.1%	0.81	1.4%
Blood No. 6	65	0.59	0.9%	0.38	0.6%	0.00	0.0%	0.34	0.5%	0.38	0.6%	0.86	1.3%
Blood No. 7	86	0.73	0.8%	0.47	0.5%	0.00	0.0%	0.44	0.5%	1.00	1.2%	1.39	1.6%
Blood No. 8	107	0.70	0.6%	0.76	0.7%	0.00	0.0%	0.70	0.6%	2.41	2.2%	2.71	2.5%

(*) Total reproducibility includes : within-capillary, between-capillary, between-run, between-day and between-lot.

④	Mean (mmol/mol)	Within-lot (*)					
		Lot No. 1		Lot No. 2		Lot No. 3	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	33	0.67	2.0%	0.62	1.9%	0.75	2.3%
Blood No. 2	34	0.72	2.1%	0.75	2.3%	0.65	1.9%
Blood No. 3	37	0.65	1.8%	0.70	1.9%	0.77	2.1%
Blood No. 4	48	0.80	1.7%	0.78	1.6%	0.70	1.5%
Blood No. 5	61	0.97	1.6%	0.60	1.0%	0.83	1.4%
Blood No. 6	65	0.76	1.2%	0.64	1.0%	0.93	1.4%
Blood No. 7	86	0.98	1.2%	0.72	0.8%	1.16	1.3%
Blood No. 8	107	1.33	1.3%	1.20	1.1%	1.25	1.2%

(*) Within-lot reproducibility includes : within-capillary, between-capillary, between-run and between-day.

⑤	Mean (%)	Within-capillary		Between-capillary		Between-run		Between-day		Between-lot		Total reproducibility (*)	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	5.2	0.04	0.9%	0.04	0.8%	0.00	0.0%	0.02	0.4%	0.04	0.8%	0.08	1.5%
Blood No. 2	5.2	0.04	0.8%	0.05	0.9%	0.00	0.0%	0.03	0.6%	0.05	0.9%	0.08	1.6%
Blood No. 3	5.5	0.05	0.8%	0.05	0.9%	0.00	0.0%	0.04	0.7%	0.04	0.7%	0.09	1.6%
Blood No. 4	6.5	0.05	0.7%	0.04	0.6%	0.02	0.3%	0.02	0.2%	0.05	0.7%	0.08	1.2%
Blood No. 5	7.7	0.05	0.6%	0.04	0.6%	0.00	0.0%	0.03	0.4%	0.01	0.1%	0.07	0.9%
Blood No. 6	8.1	0.06	0.7%	0.04	0.5%	0.00	0.0%	0.03	0.4%	0.03	0.4%	0.08	1.0%
Blood No. 7	10.1	0.06	0.6%	0.04	0.4%	0.00	0.0%	0.04	0.4%	0.10	1.0%	0.13	1.3%
Blood No. 8	11.9	0.07	0.6%	0.07	0.6%	0.00	0.0%	0.06	0.5%	0.21	1.8%	0.24	2.0%

(*) Total reproducibility includes : within-capillary, between-capillary, between-run, between-day and between-lot.

⑥	Mean (%)	Within-lot (*)					
		Lot No. 1		Lot No. 2		Lot No. 3	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	5.2	0.06	1.1%	0.07	1.3%	0.07	1.4%
Blood No. 2	5.2	0.06	1.2%	0.08	1.6%	0.06	1.2%
Blood No. 3	5.5	0.07	1.3%	0.07	1.4%	0.08	1.4%
Blood No. 4	6.5	0.07	1.1%	0.06	0.9%	0.07	1.0%
Blood No. 5	7.7	0.09	1.1%	0.06	0.8%	0.07	0.9%
Blood No. 6	8.1	0.08	0.9%	0.06	0.7%	0.09	1.1%
Blood No. 7	10.1	0.09	0.9%	0.08	0.8%	0.09	0.9%
Blood No. 8	11.9	0.12	1.0%	0.11	0.9%	0.12	1.0%

(*) Within-lot reproducibility includes : within-capillary, between-capillary, between-run and between-day.

Instrument No. 2

⑦	Mean (mmol/mol)	Within-capillary		Between-capillary		Between-run		Between-day		Between-lot		Total reproducibility (*)	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	33	0.57	1.7%	0.55	1.7%	0.00	0.0%	0.22	0.7%	0.45	1.4%	0.94	2.8%
Blood No. 2	34	0.54	1.6%	0.56	1.7%	0.00	0.0%	0.24	0.7%	0.42	1.3%	0.91	2.7%
Blood No. 3	37	0.45	1.2%	0.53	1.5%	0.00	0.0%	0.23	0.6%	0.31	0.8%	0.80	2.2%
Blood No. 4	48	0.61	1.3%	0.41	0.9%	0.37	0.8%	0.15	0.3%	0.30	0.6%	0.89	1.9%
Blood No. 5	61	0.55	0.9%	0.28	0.5%	0.00	0.0%	0.48	0.8%	0.00	0.0%	0.78	1.3%
Blood No. 6	65	0.69	1.1%	0.34	0.5%	0.00	0.0%	0.31	0.5%	0.03	0.0%	0.83	1.3%
Blood No. 7	86	0.58	0.7%	0.55	0.6%	0.00	0.0%	0.40	0.5%	0.41	0.5%	0.99	1.1%
Blood No. 8	107	0.81	0.8%	1.01	0.9%	0.00	0.0%	0.76	0.7%	1.38	1.3%	2.04	1.9%

(*) Total reproducibility includes : within-capillary, between-capillary, between-run, between-day and between-lot.

⑧	Mean (mmol/mol)	Within-lot (*)					
		Lot No. 1		Lot No. 2		Lot No. 3	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	33	0.88	2.6%	0.73	2.2%	0.84	2.5%
Blood No. 2	34	0.88	2.6%	0.65	2.0%	0.90	2.7%
Blood No. 3	37	0.87	2.4%	0.56	1.5%	0.74	2.0%
Blood No. 4	48	0.79	1.7%	0.89	1.9%	0.86	1.8%
Blood No. 5	61	0.66	1.1%	0.67	1.1%	0.98	1.6%
Blood No. 6	65	0.75	1.2%	0.77	1.2%	0.99	1.5%
Blood No. 7	86	1.03	1.2%	0.79	0.9%	0.86	1.0%
Blood No. 8	107	1.73	1.6%	1.45	1.4%	1.47	1.4%

(*) Within-lot reproducibility includes : within-capillary, between-capillary, between-run and between-day.

⑨	Mean (%)	Within-capillary		Between-capillary		Between-run		Between-day		Between-lot		Total reproducibility (*)	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	5.2	0.05	1.0%	0.05	0.9%	0.00	0.0%	0.02	0.4%	0.05	0.9%	0.09	1.7%
Blood No. 2	5.2	0.05	0.9%	0.06	1.1%	0.00	0.0%	0.02	0.4%	0.03	0.7%	0.09	1.6%
Blood No. 3	5.5	0.04	0.8%	0.04	0.8%	0.00	0.0%	0.02	0.4%	0.04	0.8%	0.08	1.4%
Blood No. 4	6.5	0.05	0.7%	0.03	0.5%	0.02	0.3%	0.02	0.4%	0.02	0.4%	0.07	1.1%
Blood No. 5	7.7	0.05	0.7%	0.03	0.3%	0.00	0.0%	0.05	0.6%	0.01	0.1%	0.08	1.0%
Blood No. 6	8.1	0.07	0.8%	0.03	0.3%	0.01	0.1%	0.03	0.3%	0.01	0.1%	0.08	0.9%
Blood No. 7	10.1	0.05	0.5%	0.05	0.5%	0.00	0.0%	0.03	0.3%	0.05	0.5%	0.10	1.0%
Blood No. 8	11.9	0.08	0.6%	0.09	0.8%	0.02	0.2%	0.07	0.6%	0.13	1.1%	0.19	1.6%

(*) Total reproducibility includes : within-capillary, between-capillary, between-run, between-day and between-lot.

⑩	Mean (%)	Within-lot (*)					
		Lot No. 1		Lot No. 2		Lot No. 3	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	5.2	0.08	1.5%	0.06	1.2%	0.08	1.6%
Blood No. 2	5.2	0.09	1.7%	0.07	1.3%	0.08	1.6%
Blood No. 3	5.5	0.06	1.2%	0.06	1.1%	0.07	1.3%
Blood No. 4	6.5	0.06	1.0%	0.06	1.0%	0.07	1.1%
Blood No. 5	7.7	0.06	0.8%	0.06	0.8%	0.10	1.2%
Blood No. 6	8.1	0.07	0.8%	0.08	0.9%	0.09	1.1%
Blood No. 7	10.1	0.10	1.0%	0.07	0.7%	0.08	0.8%
Blood No. 8	11.9	0.15	1.3%	0.14	1.2%	0.14	1.2%

(*) Within-lot reproducibility includes : within-capillary, between-capillary, between-run and between-day.

Instrument No. 3

⑪	Mean (mmol/mol)	Within-capillary		Between-capillary		Between-run		Between-day		Between-lot		Total reproducibility (*)	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	33	0.44	1.3%	0.46	1.4%	0.00	0.0%	0.30	0.9%	0.33	1.0%	0.77	2.3%
Blood No. 2	34	0.49	1.4%	0.50	1.5%	0.00	0.0%	0.32	0.9%	0.40	1.2%	0.87	2.6%
Blood No. 3	37	0.40	1.1%	0.54	1.5%	0.00	0.0%	0.29	0.8%	0.48	1.3%	0.88	2.4%
Blood No. 4	48	0.52	1.1%	0.49	1.0%	0.17	0.4%	0.21	0.4%	0.32	0.7%	0.83	1.7%
Blood No. 5	61	0.54	0.9%	0.46	0.7%	0.00	0.0%	0.42	0.7%	0.15	0.2%	0.84	1.3%
Blood No. 6	65	0.59	0.9%	0.55	0.8%	0.00	0.0%	0.28	0.4%	0.12	0.2%	0.86	1.3%
Blood No. 7	86	0.58	0.7%	0.45	0.5%	0.00	0.0%	0.31	0.4%	0.61	0.7%	1.01	1.2%
Blood No. 8	107	0.69	0.6%	0.68	0.6%	0.00	0.0%	0.53	0.5%	1.39	1.3%	1.77	1.7%

(*) Total reproducibility includes : within-capillary, between-capillary, between-run, between-day and between-lot.

(12)	Mean (mmol/mol)	Within-lot (*)					
		Lot No. 1		Lot No. 2		Lot No. 3	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	33	0.61	1.8%	0.89	2.7%	0.57	1.7%
Blood No. 2	34	0.64	1.9%	1.00	3.0%	0.59	1.7%
Blood No. 3	37	0.53	1.4%	0.94	2.6%	0.67	1.8%
Blood No. 4	48	0.72	1.5%	0.88	1.8%	0.74	1.5%
Blood No. 5	61	0.78	1.2%	0.87	1.4%	0.83	1.3%
Blood No. 6	65	0.71	1.1%	0.88	1.4%	0.96	1.5%
Blood No. 7	86	0.69	0.8%	0.76	0.9%	0.93	1.1%
Blood No. 8	107	1.09	1.0%	1.16	1.1%	1.09	1.0%

(*) Within-lot reproducibility includes : within-capillary, between-capillary, between-run and between-day.

(13)	Mean (%)	Within-capillary		Between-capillary		Between-run		Between-day		Between-lot		Total reproducibility (*)	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	5.2	0.05	0.9%	0.04	0.8%	0.00	0.0%	0.03	0.5%	0.01	0.2%	0.07	1.3%
Blood No. 2	5.2	0.05	0.9%	0.04	0.8%	0.00	0.0%	0.03	0.6%	0.03	0.6%	0.08	1.5%
Blood No. 3	5.5	0.04	0.8%	0.04	0.7%	0.00	0.0%	0.02	0.4%	0.04	0.7%	0.07	1.3%
Blood No. 4	6.5	0.05	0.8%	0.04	0.6%	0.02	0.4%	0.02	0.2%	0.03	0.4%	0.07	1.1%
Blood No. 5	7.7	0.05	0.6%	0.04	0.6%	0.00	0.0%	0.03	0.4%	0.02	0.3%	0.08	1.0%
Blood No. 6	8.1	0.06	0.7%	0.05	0.6%	0.00	0.0%	0.03	0.4%	0.01	0.1%	0.08	1.0%
Blood No. 7	10.1	0.05	0.5%	0.04	0.4%	0.00	0.0%	0.03	0.3%	0.06	0.6%	0.10	1.0%
Blood No. 8	11.9	0.06	0.5%	0.07	0.6%	0.00	0.0%	0.05	0.4%	0.13	1.1%	0.17	1.4%

(*) Total reproducibility includes : within-capillary, between-capillary, between-run, between-day and between-lot.

(14)	Mean (%)	Within-lot (*)					
		Lot No. 1		Lot No. 2		Lot No. 3	
Sample		**SD**	**CV**	**SD**	**CV**	**SD**	**CV**
Blood No. 1	5.2	0.05	1.0%	0.09	1.6%	0.06	1.2%
Blood No. 2	5.2	0.06	1.2%	0.09	1.7%	0.06	1.2%
Blood No. 3	5.5	0.05	0.9%	0.08	1.4%	0.06	1.1%
Blood No. 4	6.5	0.07	1.0%	0.08	1.2%	0.06	1.0%
Blood No. 5	7.7	0.07	0.9%	0.08	1.0%	0.07	0.9%
Blood No. 6	8.1	0.07	0.9%	0.08	1.0%	0.09	1.2%
Blood No. 7	10.1	0.06	0.6%	0.07	0.7%	0.10	0.9%
Blood No. 8	11.9	0.11	0.9%	0.11	0.9%	0.10	0.9%

(*) Within-lot reproducibility includes : within-capillary, between-capillary, between-run and between-day.

Linearity

Mixture of 2 different blood samples :

This linearity study of the CAPI 3 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; Approved Guideline".
The results for HbA_{1c} concentration (mmol/mol) and percentage (%) were analyzed using statistical tools recommended by CLSI.

2 characteristic blood samples, including a normal sample and an elevated HbA_{1c} level sample were mixed within different proportions and the mixtures were electrophoresed with the CAPI 3 Hb A1c procedure. For each mixture, samples were analyzed in triplicate.
The tests were determined to be linear within the entire range studied for HbA_{1c} hemoglobin fraction. The stated measuring range is 20 mmol/mol to 157 mmol/mol HbA_{1c} (4.0 % to 16.5 % HbA_{1c}).

Dilution of 4 different blood samples in hemolysing solution:

4 different characteristic blood samples, including 1 normal sample with HbA_{1c} concentration at 21 mmol/mol (4.1 % HbA_{1c}), 1 sample with HbA_{1c} level close to the cut-off value with HbA_{1c} concentration at 47 mmol/mol (6.4 % HbA_{1c}) and 2 elevated HbA_{1c} level samples with HbA_{1c} concentrations at 82 mmol/mol (9.6 % HbA_{1c}) and at 134 mmol/mol (14.4 % HbA_{1c}), were all serially diluted in hemolysing solution and electrophoresed with the CAPI 3 Hb A1c procedure.
The tests were determined to be linear within the entire ranges studied from 2.9 to 30.5 g/dL total hemoglobin and HbA_{1c} fraction concentration and percentage were not affected by the hemoglobin concentration of the samples.

Accuracy

The concordance study of the CAPI 3 Hb A1c procedure performed with the CAPILLARYS 3 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 HbA_{1c} concentrations (mmol/mol) and percentages (%) were analyzed using statistical tools recommended by CLSI.

The levels of HbA_{1c} were measured in 152 blood samples, including samples with normal and elevated HbA_{1c} levels, both by electrophoretic separations obtained with the CAPI 3 Hb A1c procedure on the CAPILLARYS 3 instrument and a commercially available high-performance liquid chromatography technique for HbA_{1c} quantification that is NGSP standardized.
The measured values of HbA_{1c} concentrations and percentages from both procedures were analyzed by a linear regression statistical procedure. The results of linear regression analysis are tabulated below (y = CAPI 3 Hb A1c), the sensibility and specificity of the CAPI 3 Hb A1c procedure compared to the reference procedure have been calculated using the recommended method (Wendling, 1986):

HbA_{1c}	Correlation coefficient	y-Intercept	Slope	Range of values CAPI 3 Hb A1c	Sensibility (%)	Specificity (%)
Concentration (mmol/mol)	0.999	-1.122	1.012	20 - 157	91.8	100.0
Percentage (%)	0.999	-0.142	1.014	3.9 – 16.5	94.8	100.0

Limit of blank (LOB) – Limit of detection (LOD)

The determination of the limit of blank (LOB) and the limit of detection (LOD) of the CAPI 3 Hb A1c procedure was evaluated in a study based on the Clinical and Laboratory Standards Institute (CLSI - USA) EP17-A guideline "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline".

The Limit of Blank (LOB) and Limit of Detection (LOD) were determined by assaying five samples without HbA_{1c} and five samples with low HbA_{1c}, respectively.
The results are as follows: LOB = 0.1 %, LOD = 1.2 %.

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SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES - ELECTROPHORETIC PATTERNS

Figure 1

Profil normal
Normal pattern

HbA_{1c} : 5.1 % - 33 mmol/mol

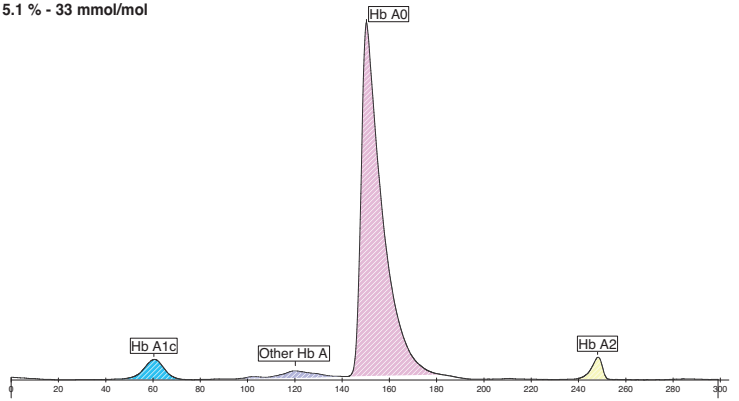
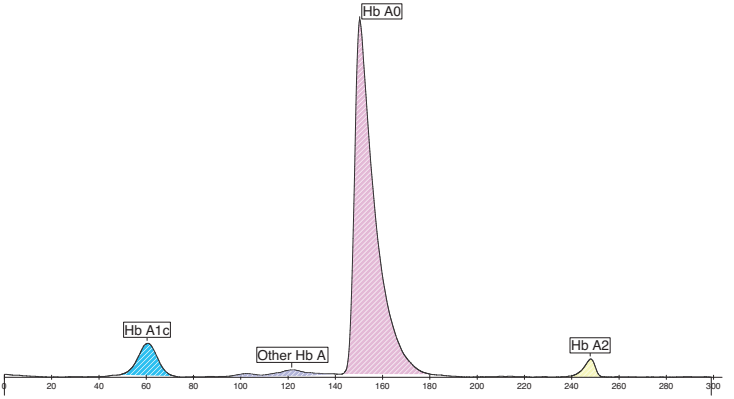


Figure 2

Profil avec HbA_{1c} augmentée
Pattern with elevated HbA_{1c} level

HbA_{1c} : 8.3 % - 68 mmol/mol



SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES - ELECTROPHORETIC PATTERNS

Figure 3

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

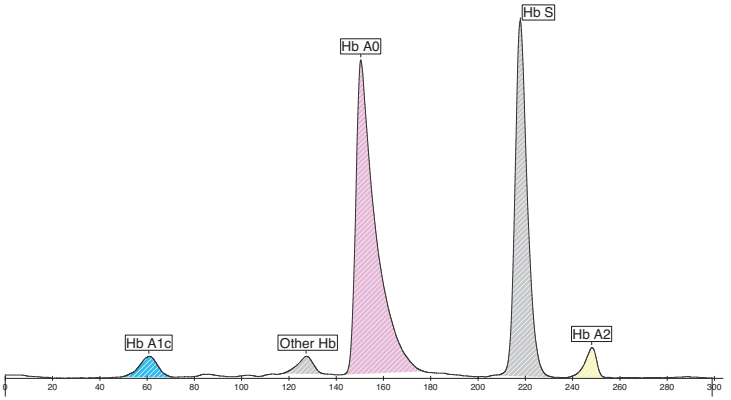
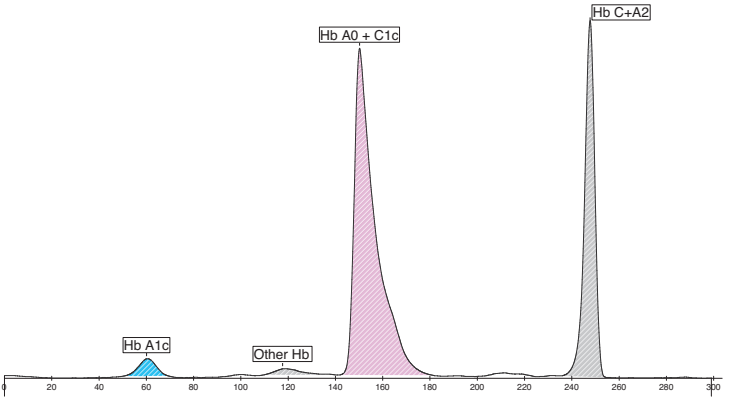


Figure 4

Profil avec variant (Hb C suspectée)
Pattern with variant (suspected Hb C)

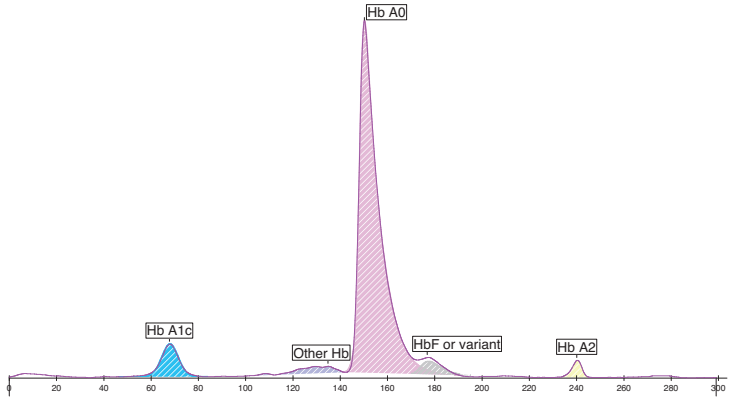


SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES - ELECTROPHORETIC PATTERNS

Figure 5

Profil avec Hb F
Pattern with Hb F



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