

sebia

MINICAP Hb A1c

Ref. 2215

IVD



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INTENDED USE

The MINICAP Hb A1c kit is designed for separation and quantification of the HbA_{1c} glycosylated fraction of hemoglobin in human blood, by capillary electrophoresis in alkaline buffer (pH 9.4) with the MINICAP FLEX-PIERCING instrument.
The MINICAP FLEX-PIERCING 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 K₂EDTA or K₃EDTA 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 MINICAP Hb A1c procedure performed with the MINICAP FLEX-PIERCING instrument has been certified by the National Glycohemoglobin Standardization Program (NGSP).

For *In Vitro* Diagnostic Use.

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 β chains. This reaction takes place during the entire life of the red blood cells. The rate of glycosylated 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 glycosylated 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} glycosylated fraction. The MINICAP 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 MINICAP 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 MINICAP FLEX-PIERCING instrument has silica capillaries functioning in parallel allowing 2 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 MINICAP 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 MINICAP Hb A1c KIT

WARNING : See the safety data sheets.

ITEMS	PN 2215
Buffer (ready to use)	2 vials, 250 mL each
Hemolysing solution (ready to use)	1 vial, 225 mL
Wash solution (stock solution)	1 vial, 25 mL
Reagent cups	1 pack of 125
Filters	3 filters
Bins for used cups	4 bins
Hemolysing solution bar code labels	5 sheets of 4 labels

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 (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 (15 to 30 °C) 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 (15 to 30 °C) 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 (15 to 30 °C).

Once the buffer vial has been opened and positioned on the MINICAP 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 (15 to 30 °C) 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 250 mL with distilled or deionized water.

After dilution, the wash solution contains an alkaline solution pH ≈ 12 .

Use

For washing the capillaries before HbA_{1c} electrophoresis.

IMPORTANT : Before filling the wash solution container, it is recommended to wash 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 (15 to 30 °C) or refrigerated (2 to 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.

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

4. REAGENT CUPS

Use

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 MINICAP FLEX-PIERCING. One reagent cup is intended for the analysis of 2 samples.

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.

5. FILTERS

Use

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

IMPORTANT : Replace all filters simultaneously upon opening a new kit. 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, wash solution and distilled or deionized water. When setting filters on MINICAP 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 (15 to 30 °C) or refrigerated (2 to 8 °C).

6. BINS FOR USED CUPS

Use

Bins intended for automated collection of used reagent cups in MINICAP FLEX-PIERCING. To place in MINICAP FLEX-PIERCING at the location intended for this purpose.

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

7. HEMOLYSING SOLUTION BAR CODE LABELS

Use

Labels to identify the tube containing the hemolysing solution (HbA1c HEMOLYSING SOLUTION).

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 MINICAP Hb A1c electrophoresis procedure performed with the MINICAP 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 MINICAP FLEX-PIERCING 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) ;
 - at least every 2 months ;
 - after having changed a capillary.

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

Use

IMPORTANT :

- For optimal use of each Hb A1c CAPILLARY Calibrator with the MINICAP 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.
- 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.
- The analysis of each calibrator must be performed according to the same procedure as described below.
- For each calibrator, place the tube (identified with the Calibrator bar code label), in position No. 28 on a MINICAP FLEX-PIERCING rotating sampler ("Control" position with centering ring).
- Pour 5 mL of MINICAP Hb A1c hemolysing solution in a hemolysing tube (identified with the hemolysing solution bar code label) without introducing air bubbles and place it in position No. 27 on the rotating sampler ("Diluent / Solution" position without any centering ring) (A message will be displayed if the tube or the hemolysing solution is missing).

IMPORTANT : Ensure the absence of foam in the tube before placing it on the rotating sampler.

- **Do not place any sample tube in positions 1 to 26 of the rotating sampler.**
- Slide the rotating sampler into the MINICAP FLEX-PIERCING instrument.
- Close the doors of the MINICAP FLEX-PIERCING 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.
- Remove the tube with calibrator from position 28 as soon as the window appears indicating to remove the tube.
- When the analysis of the first calibrator is completed, perform the analysis of the second calibrator according to the same procedure.

WARNING : After having closed the doors of the instrument, to start the analysis of the second calibrator :

- wait until the instrument has checked the absence of tube in position No. 1,
- click on the flashing button to open the "Information messages" window indicating the absence of tube in position No. 1,
- close the "Information messages" window,
- start the analysis using the "Click to run the control in position 28" button.

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 MINICAP Hb A1c electrophoresis procedure performed with the MINICAP 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 MINICAP 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 MINICAP 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 MINICAP FLEX-PIERCING instrument,
- after having changed one or both capillaries,
- after having changed the lot number of calibrators or controls.

Each control must be analyzed 3 times in position No. 28, see the package insert of the Hb A1c CAPILLARY Controls.

Quality control :

- The recommendations to analyze one of the two controls on both 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 ;
 - after the analysis of a complete rotating sampler, 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.
- Identify the tube with the specific bar code label of the control.
- The analysis of each control must be performed according to the same procedure as described below.
- For each control, place the tube (identified with the Control bar code label), in position No. 28 on a MINICAP FLEX-PIERCING rotating sampler ("Control" position with centering ring).
- Pour 5 mL of MINICAP Hb A1c hemolysing solution in a hemolysing tube (identified with the hemolysing solution bar code label) without introducing air bubbles and place it in position No. 27 on the rotating sampler ("Diluent / Solution" position without any centering ring) (A message will be displayed if the tube or the hemolysing solution is missing).

IMPORTANT : Ensure the absence of foam in the tube before placing it on the rotating sampler.

- Slide the rotating sampler into the MINICAP FLEX-PIERCING instrument.
- Close the doors of the MINICAP FLEX-PIERCING instrument, the analysis starts automatically.
- In the window which appears on the screen, select the number of analyses of the Hb A1c CAPILLARY Control to perform and validate.
- The results are then automatically considered by the software for the data analysis.
- Remove the tube with control from position 28 as soon as the window appears indicating to remove the tube.
- Perform the analysis of the second control according to the same procedure.

WARNING : After having closed the doors of the instrument, if the analysis of the second blood control is performed within the same day than that of the first blood control, start this analysis using the "Click to run the control in position 28" button. Do not place any sample tube in positions 1 to 26 of the rotating sampler.

- 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").

NOTE : 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.

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.

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 MINICAP FLEX-PIERCING, SEBIA, for capillary electrophoresis.

It is recommended to use filtered distilled or deionized water (on a filter with a porosity $\leq 0.45 \mu\text{m}$) and with a conductivity lower than $3 \mu\text{S/cm}$, which corresponds to a resistivity higher than $0.33 \text{ M}\Omega\cdot\text{cm}$.

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. MINICAP FLEX-PIERCING CAPICLEAN

Composition

The vial of CAPICLEAN concentrated solution (MINICAP FLEX-PIERCING CAPICLEAN, SEBIA, PN 2251, 25 mL) contains : proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, necessary for optimum performances.

Use

For sample probe cleaning in the SEBIA MINICAP FLEX-PIERCING automated instrument, 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 MINICAP FLEX-PIERCING CAPICLEAN, SEBIA.

WARNING : Do not use a capped tube.

IMPORTANT : For optimal use of the CAPICLEAN solution with the MINICAP FLEX-PIERCING instrument, it is necessary to use one bar code label intended to identify the 100 mm tube which contains the diluted CAPICLEAN solution.

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.

For later use, store the tube containing the diluted solution at 2 - 8 °C. It must be used within the day.

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 MINICAP FLEX-PIERCING instrument (weekly maintenance in order to eliminate adsorbed proteins from the probe).

See the SEBIA MINICAP FLEX-PIERCING instruction manual.

WARNING : Do not use a capped tube.

- Apply in a 100 mm tube 2 mL of diluted chlorinated solution previously prepared.
- Place the 100 mm tube (identified with one bar code label specific to the sodium hypochlorite solution) on the rotating sampler of MINICAP FLEX-PIERCING.
- Position a new reagent cup on the automated loading system for cups of MINICAP FLEX-PIERCING (a message will be displayed if the reagent cup is missing).
- Slide the rotating sampler into the MINICAP FLEX-PIERCING instrument.
- Close the doors of the MINICAP FLEX-PIERCING instrument, the cleaning sequence starts automatically.

IMPORTANT : For optimal use of the sodium hypochlorite solution with the MINICAP FLEX-PIERCING instrument, it is necessary to use one bar code label intended to identify the 100 mm tube which contains the solution.

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. CAPILLARIES / MINICAP WASH SOLUTION

Preparation

Each vial of the stock CAPILLARIES / MINICAP 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.

For MINICAP FLEX-PIERCING, it is convenient to dilute only 25 mL of the stock solution to 250 mL with distilled or deionized water.

Use

For washing the MINICAP FLEX-PIERCING capillaries.

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 (15 to 30 °C) or refrigerated (2 to 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.

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. MINICAP FLEX-PIERCING instrument, SEBIA, PN 1232.
2. Update HbA1c kit for MINICAP FLEX-PIERCING, SEBIA, PN 1238.
3. Rotating sampler supplied with MINICAP FLEX-PIERCING, equipped with centering rings for MINICAP FLEX-PIERCING (specific for the MINICAP Hb A1c procedure) for 13 mm diameter tubes.
4. MINICAP FLEX-PIERCING centering rings for 11 mm diameter tubes, SEBIA (27 units), PN 1612.
5. Container Kit supplied with MINICAP FLEX-PIERCING : Rinse container (to fill with distilled or deionized water) and waste container.
6. Collection tubes with 13 mm diameter and their corresponding caps (maximal length of tube with cap : 100 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 : 100 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.
7. 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 particular samples (with a volume below 1 mL), with the MINICAP FLEX-PIERCING instrument.
8. Hemolysing tubes (with 8 to 16 mm diameter and 50 to 100 mm length).
9. "AUTOMATIC LOW VOLUME" bar code labels, SEBIA (20 units), PN 9208 : labels intended to identify samples with volume less than 1 mL for an analysis using the "automatic dilution" mode.
10. "MANUAL LOW VOLUME" bar code labels, SEBIA (20 units), PN 9209 : labels intended to identify samples with volume less than 1 mL for an analysis using the "manual dilution" mode.
11. MINICAP Reagent cups / 125 (3), SEBIA, PN 2281.
12. Lids for bins for used reagent cups, SEBIA (12 units), PN 2286 : lids to close the bins containing used cups.

SAMPLES FOR ANALYSIS

Sample collection and storage

Fresh anticoagulated whole blood samples collected in tubes containing K₂EDTA or K₃EDTA 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 - 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 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 - 70 / - 80 °C.**

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

Particular cases :

IMPORTANT : The following samples must be identified with the "LOW VOLUME AUTO" or "LOW VOLUME MANUAL" bar code label ; these labels allow the instrument to apply respectively the automatic or manual dilution mode according to the sample preparation procedure. These samples must be placed on the rotating sampler at the beginning of an analysis series and the bar code of each tube must be visible in the opening of the rotating sampler. Without any bar code label, the analysis should be affected.

Analysis of samples with volume below 1 mL :

- Vortex for 5 seconds the whole blood sample and prepare the sample according to its volume following one of the 2 protocols.

1. Volume of sample comprised between 200 µL and 1 mL :

- Apply at least 200 µL of whole blood to analyze in a conical tube for control (identified with a "LOW VOLUME AUTO" bar code label) and cap the tube.
- Place the tube on the rotating sampler of the MINICAP FLEX-PIERCING instrument at the beginning of an analysis series and slide the rotating sampler into the instrument to run the analysis without any delay.
- Close the doors of the MINICAP FLEX-PIERCING instrument, the analysis starts automatically.

or

2. Volume of sample below 200 µL :

- Apply 50 µL of whole blood to analyze in a conical tube for control (identified with a "LOW VOLUME MANUAL" bar code label) and 250 µL of MINICAP Hb A1c hemolysing solution and cap the tube.
- Vortex for 5 seconds.
- Place the tube on the rotating sampler of the MINICAP FLEX-PIERCING instrument at the beginning of an analysis series and slide the rotating sampler into the instrument to run the analysis without any delay.
- Close the doors of the MINICAP FLEX-PIERCING instrument, the analysis starts automatically.

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

NOTES : The minimal volume of sample to analyze in a conical tube is 200 µL. Without any bar code label on the conical tube, the sample cannot be identified and the dilution mode (automatic or manual according to the sample preparation procedure) cannot be applied.

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. 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 cases).

PROCEDURE

The MINICAP FLEX-PIERCING instrument is a multiparameter instrument for hemoglobins analysis on parallel capillaries. The hemoglobins assay uses 2 capillaries to run the samples.

The sequence of automated steps is as follows :

- Bar code reading of rotating sampler and sample tubes (for up to 26 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 rotating sampler in positions 1 to 26 ;
- Placement of the uncapped hemolysing solution tube in rotating sampler in position 27 ;
- Placement of the Hb A1c Capillary Calibrators and Controls in rotating sampler in position 28 ;
- Placement of the rotating sampler in the MINICAP FLEX-PIERCING instrument ;
- Removal of the sample tubes after analysis ;
- Removal and closing of the bins for used cups.

PLEASE CAREFULLY READ THE MINICAP FLEX-PIERCING INSTRUCTION MANUAL.

I. PREPARATION OF MINICAP ANALYSIS

1. Switch on MINICAP FLEX-PIERCING instrument and computer.
2. In order to start the instrument, position at least one new reagent cup on the automated loading system for cups of MINICAP FLEX-PIERCING (a message will be displayed if a reagent cup is missing).
3. Set up the software, the instrument automatically starts.
4. The MINICAP Hb A1c kit is intended to run with "Hb A1c" analysis program from the MINICAP FLEX-PIERCING instrument. To select "Hb A1c" analysis program and place the MINICAP Hb A1c buffer vial in position "B2" in the instrument, please read carefully the MINICAP FLEX-PIERCING instruction manual and follow the instructions displayed on the screen.
5. Position new reagent cups on the automated loading system for cups of MINICAP FLEX-PIERCING (a message will be displayed if the reagent cups are missing).
6. **Position a bin for used cups in MINICAP FLEX-PIERCING at the location intended for this purpose.**
7. Check the fill level of the reagent vials, add reagent if necessary and empty the waste container. In the window "Check reagent levels", update the software by moving the cursor buttons.
8. The rotating sampler contains 28 positions for sample tubes :
 - Place up to 26 capped sample tubes with whole blood on the rotating sampler with specific centering rings (positions No. 1 to 26), the bar code of each tube must be visible in the opening of the rotating sampler.
 - Pour the MINICAP Hb A1c hemolysing solution in an uncapped hemolysing tube, identified with the hemolysing solution bar code label, without introducing air bubbles : 2 mL for the analysis of 1 or 2 samples, 5 mL for the analysis of 12 samples. Place this tube in position No. 27 without any centering ring on the rotating sampler ("Diluent / Solution" position).
NOTE : 5 mL of hemolysing solution applied in a 13 x 75 mm tube will allow to perform 12 analyses ; 10 mL of hemolysing solution applied in a 16 x 100 mm tube will allow to perform 24 analyses.
- IMPORTANT :** Ensure the absence of foam in the tube before placing it on the rotating sampler.
 - Place one of the 2 Hb A1c Capillary Controls in position No. 28 on the rotating sampler ("Control" position) and select the number of analyses to perform according to the indications previously described, see "Hb A1c Capillary Controls" paragraph, part "Quality control".
- IMPORTANT :** If a tube is missing in position No. 1 (sample tube), in position No. 27 (hemolysing solution tube) and in position No. 28 (blood control or calibrator tube), the analysis cannot start and a message will be displayed.
NOTE : When using a control blood or a calibrator, it is necessary to use the specific bar code label.
9. Slide the rotating sampler into the MINICAP FLEX-PIERCING instrument.
10. Close the doors of the MINICAP FLEX-PIERCING instrument, the analysis starts automatically.
11. After the analysis, remove the rotating sampler with analyzed sample tubes.
12. If necessary, take off carefully the bin containing used reagent cups, close it tightly with the corresponding lid 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. Bar codes are read on both rotating sampler and sample tubes.
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 system.

NOTE : These automated steps are described for the two first analyzed sample tubes. The electrophoretic patterns appear after about 20 minutes from the start of the analysis. For the following sample tubes, the first three steps (bar code reading, mixing and hemolysis of blood samples) are performed during the analysis of the 2 previous samples.

II. RESULT ANALYSIS

At the end of the analysis, 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 an 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 :

Warning signal / Analyzed sample	 HbA _{1c} value outside specifications for calibrators	 No detection of Hb A0 and / or HbA _{1c} fraction	 Insufficient optical density for Hb A0 fraction	"Atypical profile" (presence of an additional fraction or deletion of a normal fraction)	 HbA _{1c} value outside the expected values for controls analyzed with the Quality Control (QC) mode
Calibrators identified with bar code labels	Warning message : "analysis of the calibrator not in conformity": analyze a control blood in position 28 or repeat the calibration [in case of invalid calibration on one (or 2) capillary(ies), the capillary(ies) is(are) deactivated, the HbA _{1c} level from blood samples and controls on the concerned capillary(ies) is then not displayed]				/
Controls identified with bar code labels	/	No HbA _{1c} value displayed	/	/	With the Quality Control mode: "+", "0" or "1" identification according to the HbA _{1c} level compared to the customized values entered by the operator.
	/	Warning message: "analysis of the control not in conformity": 1. repeat the analysis using the same vial in position 28, 2. repeat the analysis with a new vial, 3. call SEBIA Technical Service if the failure is confirmed.			With the Quality Control mode, if the warning message "analysis of the control not in conformity" is displayed, repeat the calibration.
Blood sample from patient	No HbA _{1c} value displayed (when shoulder on HbA _{1c} and / or on Hb A0)	Suspect the presence of a Hb variant or a failure in the adjustment of the electrophoretic patterns, repeat the analysis for confirmation.	Warning message: "too low OD" (< 0.07) for a blood sample that is not abnormal, repeat the analysis: if the result is confirmed, the HbA _{1c} level can be reported.	"atypical profile" and "HbA1c (*)" : suspect the presence of a Hb variant.	/

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 MINICAP 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 MINICAP 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 MINICAP FLEX-PIERCING in order to store capillaries in optimal conditions.

IMPORTANT : Position at least one new reagent cup on the automated loading system for cups of MINICAP FLEX-PIERCING (a message will be displayed if a reagent cup is missing).

IV. FILLING OF REAGENT CONTAINERS

The MINICAP 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 vial 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 MINICAP 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 4768.

In addition, for high-volume testing laboratories, it is advised to analyze one of the two controls after the analysis of a complete rotating sampler, 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 MINICAP FLEX-PIERCING 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.

Hemoglobin A_{1c} normal values : ≤ 42 mmol/mol (6.0 %) (Geistanger *et al*, 2008).

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.

HbA_{1c} threshold value for diagnosis of diabetes mellitus :

Refer to the specific guidelines for the cutoff of HbA_{1c} for the diagnosis of diabetes mellitus.

For diagnosis, the ADA (American Diabetes Association) threshold of HbA_{1c} is: ≥ 6.5 %.

Interpretation

See ELECTROPHORETIC PATTERNS.

Interferences

NOTE : The common interfering factors with the MINICAP Hb A1c procedure (triglycerides, bilirubin, ascorbic acid, urea, rheumatoid factor, glybenclamide, carbamylated and acetylated hemoglobins and labile HbA_{1c}) 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 MINICAP 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 :

Interfering factor	Concentration
Triglycerides	3.07 g/dL (35.1 mM)
Bilirubin	25.8 mg/dL (442 μM)
Ascorbic acid	60 mg/dL (3.41 mM)
Urea	291 mg/dL (48.5 mM)
Rheumatoid factor	2178 IU/mL
Glybenclamide	3 mg/dL

- No interference with the MINICAP Hb A1c procedure was detected due to the presence of carbamylated hemoglobin ($\leq 14.8\%$).
- 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 ($\leq 3.0\%$).
- 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 %".
- Analysis with hemoglobin variants :
 - No interference has been observed with HbA_{1c} fraction quantification due to the presence of major abnormal hemoglobins Hb S ($\leq 40.5\%$), Hb C ($\leq 37.0\%$), Hb D ($\leq 41.0\%$) and Hb E ($\leq 24.7\%$) (except Bart's hemoglobin). 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.
 - 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 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 HbA_{1c} may not be quantified in presence of all hemoglobin variants with this method.
- The MINICAP 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,
 - for analyzing samples from patients with conditions causing shortened red blood cell survival, such as hemolytic diseases and significant acute or chronic blood loss, and,
 - for analyzing samples from patients with total hemoglobin levels of less than 2.5 or greater than 31.1 g/dL and any hemoglobinopathies that may interfere.

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 MINICAP 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 = 120) were calculated for HbA_{1c} concentration (mmol/mol) and percentage (%) for each sample, using statistical tools recommended by CLSI.

Reproducibility between lots and instruments

Eight (8) different blood samples were run using the MINICAP Hb A1c procedure in both capillaries of 3 different MINICAP FLEX-PIERCING instruments and with 3 lots of MINICAP Hb A1c kits. 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). In this study, each blood sample was analyzed on both capillaries from each instrument, including 60 runs over 10 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 HbA_{1c} concentrations (in mmol/mol) and percentages.

	Mean (HbA _{1c} concentration - mmol/mol)	Within-run reproducibility		Total reproducibility	
		CV min (%)	CV max (%)	Total CV min (%)	Total CV max (%)
Sample No. 1	33	0.9	3.5	0.9	3.5
Sample No. 2	36	1.1	4.0	1.2	4.0
Sample No. 3	37	0.0	3.0	1.4	3.3
Sample No. 4	47	1.2	2.6	1.3	2.6
Sample No. 5	63	0.7	1.6	0.7	2.2
Sample No. 6	76	0.0	1.4	0.0	1.4
Sample No. 7	87	0.3	1.3	0.3	1.4
Sample No. 8	110	0.4	1.4	0.6	2.2
CV (%) ranges		0.0	4.0	0.0	4.0

	Mean (% HbA _{1c})	Within-run reproducibility		Total reproducibility	
		CV min (%)	CV max (%)	Total CV min (%)	Total CV max (%)
Sample No. 1	5.2	0.9	2.0	0.9	2.2
Sample No. 2	5.4	0.9	2.1	0.9	2.1
Sample No. 3	5.5	0.5	2.0	0.7	2.1
Sample No. 4	6.4	0.5	1.9	0.8	1.9
Sample No. 5	7.9	0.7	1.4	0.8	1.6
Sample No. 6	9.1	0.0	1.1	0.0	1.1
Sample No. 7	10.1	0.6	1.1	0.6	1.2
Sample No. 8	12.3	0.4	1.2	0.6	1.8
CV (%) ranges		0.0	2.1	0.0	2.2

Reproducibility within the same capillary and between capillaries from the same instrument

Eight (8) different blood samples were run using the MINICAP Hb A1c procedure in both capillaries of the same MINICAP FLEX-PIERCING instrument and with 1 lot of MINICAP Hb A1c kit. 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). In this study, each blood sample was analyzed on both 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 HbA_{1c} concentrations (in mmol/mol) and percentages are summarized in the following tables.

For reproducibility within the same capillary, maximal CV's have been calculated for each blood sample from pooled data obtained on each capillary.

	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4	Sample No. 5	Sample No. 6	Sample No. 7	Sample No. 8
Mean (HbA _{1c} concentration - mmol/mol)	33	34	38	46	62	75	87	106
Within-run reproducibility (CV %)	1.7	1.9	1.7	1.1	0.8	1.2	0.8	1.2
Within-capillary reproducibility (CV %)	2.5	2.8	2.4	1.3	0.8	1.2	1.0	0.9
Between-run reproducibility (CV %)	0.0	1.4	0.3	0.5	0.0	0.0	0.4	0.0
Between-day reproducibility (CV %)	1.3	0.0	0.6	0.5	0.0	0.6	0.3	0.2
Total (CV %)	2.2	2.3	1.8	1.3	0.8	1.3	1.0	1.2

	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4	Sample No. 5	Sample No. 6	Sample No. 7	Sample No. 8
Mean (% HbA _{1c})	5.2	5.2	5.7	6.4	7.8	9.0	10.1	11.9
Within-run reproducibility (CV %)	1.0	1.1	1.0	0.9	1.1	0.9	0.7	1.1
Within-capillary reproducibility (CV %)	1.4	1.4	1.5	1.1	0.7	0.8	0.8	0.9
Between-run reproducibility (CV %)	0.0	0.3	0.7	0.0	0.0	0.0	0.3	0.0
Between-day reproducibility (CV %)	0.7	0.0	0.5	0.6	0.2	0.4	0.3	0.1
Total (CV %)	1.2	1.1	1.3	1.1	1.1	1.0	0.8	1.1

Linearity

The linearity of the MINICAP 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 HbA_{1c} concentration (mmol/mol) and percentage (%) were analyzed using statistical tools recommended by CLSI.

Two characteristic blood samples, including a normal sample with HbA_{1c} concentration at 29 mmol/mol (4.8 % HbA_{1c}) and an elevated HbA_{1c} level sample with HbA_{1c} concentration at 127 mmol/mol (13.8 % HbA_{1c}) were mixed within different proportions and the dilutions were electrophoresed with the MINICAP Hb A1c procedure. For each dilution, samples were analyzed in duplicate. The tests were determined to be linear within the entire ranges studied for HbA_{1c} hemoglobin fraction until a maximum concentration of about 127 mmol/mol (between 4.8 and 13.8 % HbA_{1c}).

In addition, 3 different characteristic blood samples, including a normal sample with HbA_{1c} concentration at 31 mmol/mol (5.0 % HbA_{1c}), a sample with HbA_{1c} level close to the cut-off value at 46 mmol/mol (6.3 % HbA_{1c}) and an elevated HbA_{1c} level sample with HbA_{1c} concentration at 79 mmol/mol (9.3 % HbA_{1c}), were all serially diluted in hemolysing solution and electrophoresed with the MINICAP Hb A1c procedure. The tests were determined to be linear within the entire ranges studied from 2.5 to 31.1 g/dL total hemoglobin and HbA_{1c} fraction concentration and percentage were not affected by the hemoglobin concentration of the samples.

Accuracy - Internal correlation

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

NOTE : The results presented below have been obtained from 1 internal accuracy study. The analyzed blood samples were provided by 6 laboratories in France, the Netherlands and USA.

All the samples were exactly treated the same way with both techniques and followed the same guidelines in regards to sample integrity.

The levels of HbA_{1c} were measured in 101 blood samples, including 28 samples with normal HbA_{1c} levels and 73 samples with elevated HbA_{1c} levels, both by electrophoretic separations obtained with MINICAP Hb A1c procedure with the MINICAP FLEX-PIERCING instrument and a commercially available capillary electrophoresis 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 obtained results have demonstrated a perfect correlation between both procedures for HbA_{1c} quantification.

The results of linear regression analysis are tabulated below (y = MINICAP Hb A1c), the sensibility and specificity of the MINICAP 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 MINICAP Hb A1c	Sensibility (%)	Specificity (%)
Concentration (mmol/mol)	0.998	1.262	0.985	29 - 122	100.0	96.4
Percentage (%)	0.998	0.165	0.982	4.8 - 13.3	100.0	96.4

Accuracy - External correlations

The following concordance studies of the MINICAP Hb A1c procedure performed with the MINICAP FLEX-PIERCING instrument were each 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.

NOTE : The results presented below have been obtained from 2 external accuracy studies performed in 2 hospital laboratories located in the USA.

All the samples were exactly treated the same way with both techniques and followed the same guidelines in regards to sample integrity.

In study No. 1, the levels of HbA_{1c} were measured in 126 blood samples, including 53 samples with normal HbA_{1c} levels and 73 samples with elevated HbA_{1c} levels, both by electrophoretic separations obtained with MINICAP Hb A1c procedure with the MINICAP FLEX-PIERCING instrument and a commercially available capillary electrophoresis 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 obtained results have demonstrated a perfect correlation between both procedures for HbA_{1c} quantification.

The results of linear regression analysis are tabulated below (y = MINICAP Hb A1c), the sensibility and specificity of the MINICAP 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 MINICAP Hb A1c	Sensibility (%)	Specificity (%)
Concentration (mmol/mol)	0.998	- 0.396	0.996	29 - 125	91.8	94,3
Percentage (%)	0.998	- 0.032	0.997	4.8 - 13.6	90.4	98.1

In study No. 2, the levels of HbA_{1c} were measured in 140 blood samples, including 54 samples with normal HbA_{1c} levels and 86 samples with elevated HbA_{1c} levels, both by electrophoretic separations obtained with MINICAP Hb A1c procedure with the MINICAP FLEX-PIERCING instrument and a commercially available capillary electrophoresis 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 obtained results have demonstrated a perfect correlation between both procedures for HbA_{1c} quantification.

The results of linear regression analysis are tabulated below (y = MINICAP Hb A1c), the sensibility and specificity of the MINICAP 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 MINICAP Hb A1c	Sensibility (%)	Specificity (%)
Concentration (mmol/mol)	0.998	- 0.316	1.023	29 - 119	98.8	96.3
Percentage (%)	0.998	- 0.057	1.019	4.8 - 13.1	98.8	96.3

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SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES - ELECTROPHORETIC PATTERNS

Figure 1

Profil normal
Normal pattern

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

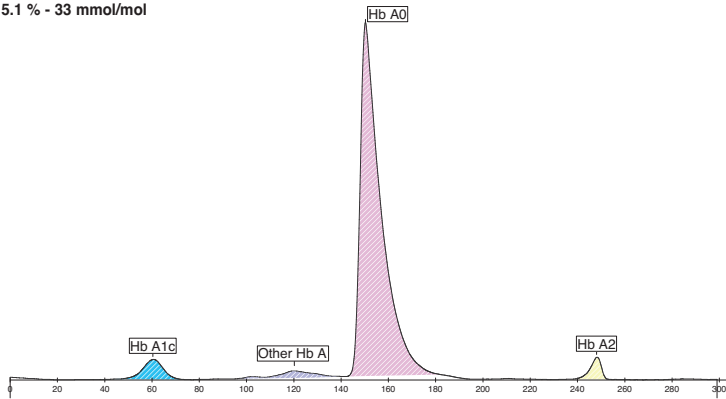
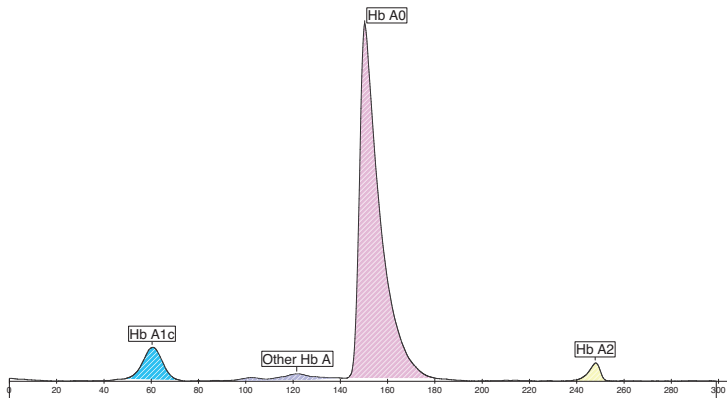


Figure 2

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)

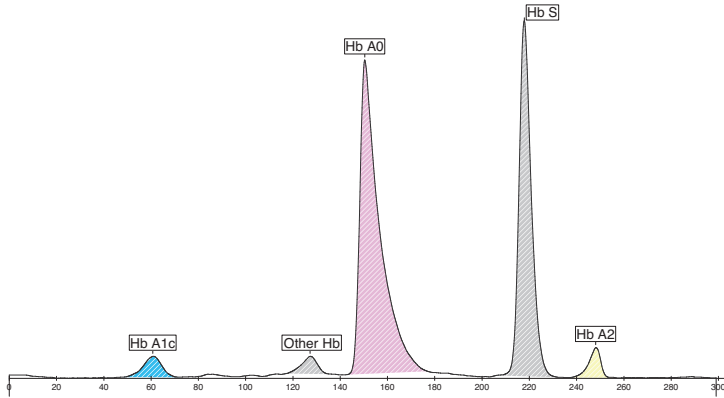
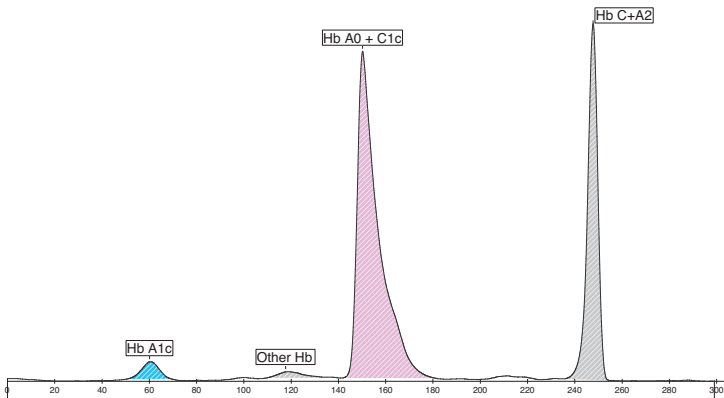


Figure 4

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

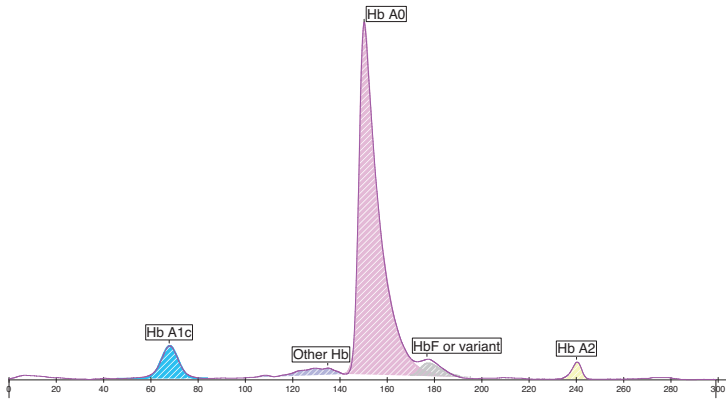


SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES - ELECTROPHORETIC PATTERNS

Figure 5

Profil avec Hb F
Pattern with Hb F



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