HYDRAGEL 7 Hb A₁c  
Ref. 4107  

HYDRAGEL 15 Hb A₁c  
Ref. 4127
INTENDED USE

The HYDRAGEL 7 Hb A1c and HYDRAGEL 15 Hb A1c kits are designed for separation and quantification of the Hb A1c glycated fraction of hemoglobin by electrophoresis on acidic buffered (pH 5.9) agarose gels. They are used in conjunction with the semi-automated HYDRASYS instrument. After migration, the gel plates are dried and directly scanned at 420 nm. Using Hb A1c calibrators, densitometry provides accurate relative quantification of Hb A1c fraction. The use of a specific software adapted to the Hb A1c allows to obtain conformed values to NGSP (National Glycohemoglobin Standardization Program).

Each agarose gel is intended to run:
• 7 samples in the HYDRAGEL 7 Hb A1c kit,
• 15 samples in the HYDRAGEL 15 Hb A1c kit.

For In Vitro Diagnostic Use. The HYDRAGEL 7 / 15 Hb A1c has completed the NGSP certification.

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 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.

The electrophoretic mobility of hemoglobin on acidic buffered (pH 5.9) agarose gel depends on hemoglobin affinity for the gel and its net electric charge.

The hemoglobin A0, with its N-terminal valin residue, has a high affinity for the sulfate groups of the gel. Its mobility is thus slowed down. The glycated hemoglobin A1c cannot combine because of the blocking effect of the attached sugar.

Electrophoresis allows separation of the fractions according to the mobility of hemoglobin A1c and affinity of hemoglobin A0 for the gel.

The natural coloration of glycated hemoglobin allows quantification of unstained electrophoregrams by densitometry.

For a better storage, the gel can be stained with amidoblack after densitometry.

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 7 Hb A1c AND HYDRAGEL 15 Hb A1c KITS

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 4107</th>
<th>PN 4127</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose Gels (ready to use)</td>
<td>10 gels</td>
<td>10 gels</td>
</tr>
<tr>
<td>Buffered Strips (ready to use)</td>
<td>10 packs of 2 each</td>
<td>10 packs of 2 each</td>
</tr>
<tr>
<td>Hemolysing Solution (ready to use)</td>
<td>1 vial, 24 mL</td>
<td>1 vial, 24 mL</td>
</tr>
<tr>
<td>Applicators (ready to use)</td>
<td>1 pack of 10 (7 teeth)</td>
<td>1 pack of 10 (15 teeth)</td>
</tr>
<tr>
<td>Filter Papers - Thin</td>
<td>1 pack of 10</td>
<td>1 pack of 10</td>
</tr>
</tbody>
</table>

FOR OPTIMAL RESULTS

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

PLEASE READ THE PACKAGE INSERT CAREFULLY.

1. AGAROSE GELS

Preparation
Agarose gels are ready to use. Each gel contains: agarose, 1.2 g/dL ; acidic buffer pH 5.9 ± 0.1 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
Support medium for hemoglobin electrophoresis.

Storage, stability and signs of deterioration
Store the gels horizontally in the original protective packaging at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the kit package or gel package label. (The arrow on the front of the kit box must be pointing upwards). Avoid storage close to a window or to a heat source. Avoid important variation of temperature during storage.

DO NOT FREEZE.

Discard when:
(i) crystals or precipitate form on the gel surface or the gel texture becomes very soft (all these result from freezing the gel),
(ii) bacterial or mold growth is indicated,
(iii) abnormal quantity of liquid is present in the gel box (as a result of buffer exudation from the gel due to improper storage conditions).

2. BUFFERED STRIPS

Preparation
Buffered sponge strips are ready to use. Each contains: acidic buffer, pH 5.7 ± 0.2.

Use
The buffered strips function as electrophoresis buffer reservoir and ensure contact between the gel and electrodes.

Storage, stability and signs of deterioration
Store the buffered strips horizontally in the original protective packaging at room temperature or refrigerated. (The arrow on the front of the kit box must be pointing upwards).

They are stable until the expiration date indicated on the kit package or buffered strips package label.

DO NOT FREEZE.

Discard buffered strips if the package is opened and the strips dry out.
3. HEMOLYSING SOLUTION
Preparation
Hemolysing Solution is ready to use. It is an acidic buffer with additives, nonhazardous at the concentrations used, necessary for optimum performance.

Use
To hemolyze red blood cells and to remove labile glycated hemoglobins.

Storage, stability and signs of deterioration
Store Hemolysing Solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or Hemolysing Solution vial label. Discard Hemolysing Solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

4. APPLICATORS
Use
Precut, single use applicators for sample application.

Storage
Store applicators in a dry place at room temperature or refrigerated.

5. FILTER PAPERS
Use
Single use absorbent pads for blotting excessive moisture off the gel surface before sample application.

Storage
Store the thin filter papers in a dry place at room temperature or refrigerated.

REAGENTS REQUIRED BUT NOT SUPPLIED IN THE KITS
1. Hb A1c CALIBRATORS
Composition
Hb A1c Calibrators 1 and 2 (SEBIA, PN 4754) are obtained from pools of human blood samples. The bloods are in a stabilized lyophilised form.

Application
The Hb A1c Calibrators are designed for the calibration of electrophoretic quantification of human glycated hemoglobin A1c according to the NGSP requirements with HYDRAGEL 7 / 15 Hb A1c procedure.

For calibration, it is necessary to include one track of Hb A1c Calibrator 1 and one track of Hb A1c Calibrator 2 into each run of samples on HYDRAGEL 7 / 15 Hb A1c gels.

Procedure
Reconstitute each Hb A1c Calibrator vial with exactly 0.5 mL of distilled or deionized water. Allow to stand for 5 minutes and mix gently (avoid formation of foam).

After reconstitution, each calibrator must be used as follows: Hemolyze 40 µL reconstituted calibrator with 160 µL Hb A1c Hemolysing Solution, vortex for 10 seconds minimum, incubate for 5 minutes at room temperature and use it immediately.

IMPORTANT: Never incubate the Hb A1c Calibrators with samples.

Storage, stability and signs of deterioration
Store the lyophilised Hb A1c Calibrators refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the box or vial labels. The reconstituted calibrators should be used immediately due to the risk of microbial contamination or denaturation. The reconstituted calibrators may also be frozen (in aliquots) and stored at -20 °C for 3 months maximum. Use thawed Hb A1c Calibrators without any delay.

NOTE: During transportation, the Hb A1c Calibrators can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

No test method can provide an absolute assurance of the absence of HIV, hepatitis B and C or other infectious agents. Therefore, handle the Hb A1c Calibrators as a hazardous biological material. These calibrators were found negative:
- against hepatitis B surface antigen;
- for antibody to HCV;
- for antibody to HIV1 and HIV2.

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

Use
To wash red blood cells.

Storage, stability and signs of deterioration
Store saline at room temperature or refrigerated. Discard after 3 months or if it changes its appearance, e.g., becomes cloudy due to microbial contamination.
OPTIONAL REAGENTS FOR GEL STORAGE

1. AMIDOBLACK STAIN (SEBIA, PN 4554)

Preparation
The amidoblack concentrated stain is a viscous solution which may gelify. The integrity of the stock staining solution is not altered by the increase in viscosity or solidification.

In all cases, to obtain a perfect reconstitution of the stain, we advise you to respect the following procedure:
1. Add 15 mL of stain diluent to the concentrated amidoblack vial.
2. Close carefully the vial.
3. Shake very vigorously the vial during approximately 5 seconds.
4. Pour this solution in the container for staining solution processing.
5. Repeat this step twice, three times if necessary.
6. Pour the remaining diluent in the container and complete the volume to 300 mL with distilled or deionized water.

The staining solution is ready to use.

After dilution, the working staining solution contains: acid solution pH ≈ 2; amidoblack, 0.4 g/dL; ethylene-glycol, 6.7 %; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: Harmful if swallowed.

Use
For optional staining of the glycated hemoglobins separated on gels after densitometric scanning for electrophoretic patterns collection.

Storage, stability and signs of deterioration
Store both stock and working staining solutions at room temperature or refrigerated in closed containers to prevent evaporation. Stock staining solution is stable until the expiration date indicated on the kit package or staining vial labels. Working staining solution is stable for 1 month. Its stability may be extended for 3 months if the working solution is refrigerated. The closed container must be stored refrigerated immediately after each use.

Do not store the working staining solution close to a heat source.

Store the stock staining solution diluted at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or staining solution diluted vial labels. DO NOT FREEZE.

2. DESTAINING SOLUTION

Preparation
Each vial of stock Destaining Solution (SEBIA, PN 4540, 10 vials, 100 mL each) to be diluted up to 100 liters with distilled or deionized water. It is convenient to dilute only 5 mL of the stock solution to 5 liters, the volume of the destaining solution container. After dilution, the working destaining solution contains: citric acid, 0.05 g/dL.

Use
For destaining, that is removal of excess and background stain from the gels.

For rinsing of the staining compartment after wash step.

To neutralize the acidity of the destaining solution, pour 15 mL of a 50 % solution of Sodium Hydroxide, into the empty waste container.

Storage, stability and signs of deterioration
Store the stock destaining solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or destaining solution vial labels. DO NOT FREEZE. Working destaining solution is stable for one week at room temperature in a closed bottle. Do not add any sodium azide.

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

To prevent microbial proliferation in the diluted destaining solution to be stored more than one week, add 5 µL/dL of ProClin 300. Working destaining solution added with ProClin is stable in a closed bottle at room temperature or refrigerated until the expiration date indicated on the kit package or destaining solution vial labels.

3. HYDRASYS WASH SOLUTION

Preparation
Each vial of the stock HYDRASYS Wash Solution (SEBIA, PN 4541, 10 vials, 80 mL each) to be diluted up to 5 liters with distilled or deionized water. After dilution, the working wash solution contains: alkaline buffer pH 8.8 ± 0.3; sodium azide.

WARNING: The stock wash solution contains 0.625 % sodium azide. Do not ingest! If ingested, consult physician immediately! Sodium azide may lead to formation of explosive or toxic compounds when in contact with acids, lead or copper. Always flush with a large quantity of water when disposing.

Use
It serves for cleaning of the HYDRASYS Staining Compartment. Use periodically, e.g., if the instrument is used daily, wash the staining compartment weekly.

See the package insert for directions to use.

Storage, stability and signs of deterioration
Store the stock and working wash solutions in closed containers at room temperature or refrigerated. They are stable until the expiration date indicated on the wash solution vial label.

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

EQUIPMENT AND ACCESSORIES REQUIRED BUT NOT SUPPLIED

1. HYDRASYS System SEBIA, PN 1210 or PN 1211.
2. Wet Storage Chamber, PN 1270, supplied with HYDRASYS system.
3. Pipettes: 10 µL, 100 µL and 200 µL.
4. Densitometer capable of scanning 82 x 51 mm or 82 x 102 mm gels at 420 nm or with a violet filter, e.g., HYRYS SEBIA (≥ VS 2.50). Refer to manufacturer’s instructions for operation and calibration procedures.
5. Gel carrier 0 for densitometry, SEBIA, PN 1939.
6. Gel holder for half gels, SEBIA, PN 1278.
SAMPLES FOR ANALYSIS

Sample collection and storage
Fresh anticoagulated blood samples are recommended for analysis. Anticoagulants and collection tubes containing EDTA, citrate or heparin are suitable; avoid those with iodoacetate. Blood must be collected according to established procedures used in clinical laboratory testing. If needed, store blood samples or washed red blood cells at 2 to 8 °C for up to 5 days.

Sample preparation
  • Mix the collection tube before taking the blood to prepare.
  • Centrifuge anticoagulated blood at 5,000 rpm for 5 minutes.
  • Discard the plasma.
  • Wash the red blood cells (RBC) 2 times with 10 volumes of saline; great care must be taken when processing volumes of red blood cells smaller than 15 µL.
  • Discard the excess of saline over the red blood cells pellet and vortex them before taking 15 µL to hemolyze.
  • Hemolyze exactly 15 µL packed red cells with 25 µL saline and 160 µL Hemolysing Solution.
  • Vortex and incubate the mixture in a water-bath at 45 °C for 5 minutes or at 37 °C for 15 minutes. The analysis must be performed on the hemolyzate within one hour.

NOTE: During sample preparation, it is important to pipette with precision the different volumes of RBC, saline and Hemolysing Solution.

HYDRAGEL 7 / 15 Hb A1c procedure is performed on packed red blood cells, this analysis is independent from the concentration of total hemoglobin in sample.

WARNING: The procedure to prepare Hb A1c Calibrators 1 and 2 is different than that for blood samples. Read carefully Hb A1c Calibrators preparation, see REAGENTS REQUIRED BUT NOT SUPPLIED.

PROCEDURE

The HYDRASYS system is a semi-automated multi-parameter instrument. The automated steps include processing of HYDRAGEL agarose gels in the following sequence: sample application, electrophoretic migration, drying, developing and final drying. The manual steps include handling samples and gels, and setting up the instrument for operation. READ CAREFULLY HYDRASYS INSTRUCTION MANUAL.

I. MIGRATION SET-UP

1. Switch on HYDRASYS.
2. Place one sample applicator on a flat surface with the well numbers in the right-side-up position (Fig. 1).
   IMPORTANT: It is necessary to apply Hb A1c Calibrators 1 and 2 into wells indicated below. Respect the position of the calibrators to fit the software program in order to perform the NGSP calibration.
   - Apply 10 µL Hb A1c Calibrator 1 in well N° 1 and 10 µL Hb A1c Calibrator 2 in well N° 2.
   - Apply 10 µL hemolyzed sample in the other wells. Load the applicator within 2 minutes.
   - Place the applicator into the wet storage chamber with the teeth up (handle it by the plastic tooth protection frame).
   - Let the samples diffuse into the teeth for 5 minutes after the last sample application.

   See wet chamber package insert for further details.
3. Open the lid of the Migration Module and raise the electrode and applicator carriers.
   WARNING: Never close the lid while the carriers are raised!
4. Select «7/15 Hb A1c» migration program from the instrument menu (left side of the keyboard).
5. Remove buffered strips from the package; handle them by the plastic ends. Engage the punched ends of the strip's plastic backing to the pins on the electrode carrier; the strip's plastic backing must face the carrier (Fig. 2).
6. Unpack the HYDRAGEL plate.
   - Roll quickly and uniformly one thin filter paper onto the gel surface to absorb the excess of liquid. Remove the paper immediately.
   WARNING: Do not leave the filter paper for too long contact with the gel to avoid its dehydration.
   - Pool 120 µL distilled or deionized water for HYDRAGEL 7 Hb A1c or 200 µL for HYDRAGEL 15 Hb A1c, on the lower third of the frame printed on the Temperature Control Plate of the migration module.
   - Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame (Fig. 3).
   - Bend the gel and ease it down onto the water pool (Fig. 3). Ensure that no air bubbles are trapped, water is spread underneath the entire gel plate and the gel is lined up with the printed frame.
7. Lower both carriers down. In this position the buffered strips do not touch the gel. DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.
8. Remove the applicator from the wet chamber. Handle it by the protection frame.
   -Snap off the applicator teeth's protection frame.
   -Place the applicator into position N° 9 on the carrier.
   IMPORTANT: The numbers printed on the applicator must face the operator (Fig. 4).
9. Close the lid of the migration module.
10. Start the procedure immediately by pressing the green arrow «START» key on the left side of the keyboard.
    IMPORTANT: Make sure that the ventilation air inlet on the right side of the instrument is not blocked.

MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

The two carriers are lowered so that buffered strips and applicator contact the gel surface.

Sample applicator carrier rises up.

Migration is carried out under 100 V constant, until 37 Vh have accumulated at 23 °C controlled by Peltier effect (for about 20 minutes).

The electrode carrier rises to disconnect the electrodes.

The temperature of the control plate rises to 65 °C for 15 minutes to dry the gel.

The control plate is cooled down; when it reaches 50 °C, an audible beep signals that the migration module lid unlocks. The plate temperature remains at 50 °C until the lid is opened. Then, the temperature keeps decreasing until it reaches 20 °C (in less than 5 minutes) after which a new migration run may start.

NOTE: The migration module lid remains closed during all migration steps.
II. SCANNING OF UNSTAINED GELS

1. Open the lid.
2. Remove the applicator and discard.
3. Raise both carriers, remove the buffered strips by their plastic ends and discard.
4. Remove the dried gel film for densitometric scanning.
5. Densitometric scanning must be performed only with HYRYS SEBIA DENSITOMETER AND GEL CARRIER 0 and specific Hb A1c (NGSP) software.
6. Clean a flat surface or a glass plate using a wet tissue paper with alcohol.
7. Place the film (gel side) on the clean and dry flat surface and clean the back side (the plastic support side) of the dry film with an alcohol wet tissue paper.
8. The back side of the gel must be perfectly clean and free of fingers prints.
9. Position the gel on the gel carrier with A0 fraction at level 20 mm on the carrier (Fig. 5).
10. Scan using the densitometer with a violet filter or at 420 nm without any delay.

WARNING: Do not store the gel before scanning to avoid crystallisation.
11. Wipe the electrodes and the plate with a wet tissue paper.
12. After densitometric scanning, make sure the electrophoretic patterns have been correctly analyzed by the scanning software. The Hb A1c percent is automatically calculated.

III. GEL STAINING (optional)

After densitometric scanning, to improve the detection of some abnormal hemoglobins, and/or low level fractions such as Hb F, or to detect test failures, such as hemolysate preparation, application or migration, it is recommended to stain the gel with amidoblack. Moreover, the amidoblack staining allows to improve the gel’s storage.

IMPORTANT: Do not scan the amidoblack stained gel.
1. Open the Gel Holder. Lay it flat and position the dried gel (with gel side facing up) into the grooves of the two rods and close the holder. Make sure that the film is correctly positioned inside the holder (Fig. 6).
2. Place the gel holder into the Gel Processing / Staining Module.

IMPORTANT: Before starting the gel processing / staining program, check the following:
- the staining container is filled with 300 mL of staining solution;
- the destaining container contains at least 1 liter of destaining solution;
- the waste container is empty.
For reagent line connection: refer to the information displayed on the screen of the instrument (select key: REAGENT LINES).

IMPORTANT: Do not forget to block up the unused lines.
3. Select "PROT./B1-B2/Hb" staining program from the instrument menu and start the run by pressing the "START" key (green arrow on the right side of the keyboard).

During staining, destaining and drying steps, the compartment remains locked.
After cooling step, an audible beep signals that the compartment unlocks (the ventilation is maintained until the gel holder is removed).
4. Remove the gel holder from the compartment, open it and remove the dried gel.
5. If needed, clean the back side (the plastic support side) of the dry film with a damp soft paper.

WARNING: Do not scan the amidoblack stained gel.

RESULTS

Quality Control
It is advised to include an assayed control blood or a normal blood sample into each run of samples.

Values
Densitometer scanning yields relative concentrations (percentages) of Hb A1c fraction.
Normal values (mean ± 2 SD) for Hb A1c using the HYDRAGEL 7 / 15 Hb A1c method are:

\[ \text{Hb A}1c = 4 - 6 \% \]

The Hb A1c values \( \leq 6.0 \% \) are generally considered as normal and \( > 6.0 \% \) as elevated.

It is recommended each laboratory establishes its own normal values.

Interpretation
The measurement of Hb A1c is accepted as a way of monitoring long-term glucose control during treatment of patients with diabetes mellitus. However, this test has not been found reliable in the diagnosis of diabetes mellitus.

A normal hemolysate presents three fractions:
- The most cathodic corresponds to the minor glycated hemoglobins A1a and A1b (and eventually Hb F).
- The intermediary fraction corresponds to hemoglobin A1c.
- The most anodic is the main fraction containing A0 and A2 hemoglobins.

Special Cases and Limitations
Due to the resolution and sensitivity limits of electrophoresis, it is possible that some abnormal hemoglobins may interfere with this method. Fetal hemoglobin migrates between fractions A1a + A1b and A1c when its concentration is \( \geq 1 \% \). Due to its migration proximity to A1c, Hb F at elevated levels (\( \geq 7 \% \)) usually found in infants, pregnant women and \( \beta \)-thalassemic patients, yields falsely elevated levels of hemoglobin A1c.
Abnormal hemoglobins S0 and C0 migrate more anodically than A0. In such cases, only the relative percent of hemoglobin A1c is calculated relative to hemoglobin A0 while eliminating the abnormal fractions. Presence of abnormal hemoglobin may lead to falsely decreased levels of the glycated hemoglobin concentration. However, the values represent a useful relative follow up index for the same patient. Individuals with recent significant blood loss exhibit falsely low Hb A1c values due to a higher fraction of young erythrocytes. Abnormal life span of red blood cells, as found in hemolytic anemias, polycythemia, Hb S or postsplenectomy, may effect the levels of Hb A1c. However, the values represent a useful relative follow up index for the same patient. The glycated hemoglobin levels fall considerably during pregnancy. By mid-third trimester, they are nearly 2 % lower than the non-pregnancy levels. Samples not properly stored (see SAMPLES FOR ANALYSIS) may give falsely elevated levels of Hb A1c due to continuous uptake of glucose by the red blood cells.


This analysis is independent from the concentration of total hemoglobin in sample.

### Migration patterns

![Migration patterns diagram]

### Troubleshooting

Call 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 informations on waste products elimination are available from the Technical Service of the supplier.

### PERFORMANCE DATA

SEBIA’s HYRYS densitometer was used for all densitometric evaluations.

The individual values, means, SD and CV are shown below. In overall, the results indicate a very good reproducibility for all the aspects tested of the HYDRAGEL 15 Hb A1c procedure for the quantification of hemoglobin A1c; the CV’s range from 1.3 and 3.9 % (2.6 % being the mean CV value).

### Reproducibility within run

Four blood samples were electrophoresed on four different HYDRAGEL 15 Hb A1c gels from the same lot. The four analyzed samples were: sample A with normal Hb A1c level, sample B with elevated Hb A1c level, Hb A1c Calibrators 1 and 2 (SEBIA). Each gel contained 15 tracks of each sample. The electrophoregrams were evaluated by densitometry. The following table shows the means of hemoglobin A1c, SD and CV (%) calculated for each sample from the 15 tracks.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>MEAN (%)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.8</td>
<td>0.09</td>
<td>1.8</td>
</tr>
<tr>
<td>B</td>
<td>9.5</td>
<td>0.16</td>
<td>1.7</td>
</tr>
<tr>
<td>Hb A1c Calibrator 1</td>
<td>4.3</td>
<td>0.12</td>
<td>2.8</td>
</tr>
<tr>
<td>Hb A1c Calibrator 2</td>
<td>9.4</td>
<td>0.12</td>
<td>1.3</td>
</tr>
</tbody>
</table>

In addition, none of the individual values of Hb A1c in the normal blood sample and calibrator 1 was falsely elevated and none of the individual values of Hb A1c in the elevated sample and calibrator 2 showed normal values.
Reproducibility between runs

Four blood samples were run during 20 following days on HYDRAGEL 15 Hb A1c gels from the same lot, two gels per day. The four analyzed samples were: sample C with normal Hb A1c level, sample D with elevated Hb A1c level, Hb A1c Calibrators 1 and 2 (SEBIA). Each gel contained 3 tracks of each sample. The means, SD and CV were calculated for the Hb A1c fraction from each blood sample. The electrophoregrams were evaluated by densitometry and the results were essentially the same for all samples. The following table shows for hemoglobin A1c, the range of SD and CV representing all samples and a mean CV calculated from the pooled CV's for all samples.

<table>
<thead>
<tr>
<th>Hb A1c</th>
<th>SD</th>
<th>CV (%)</th>
<th>MEAN CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.13 – 0.29</td>
<td>1.4 – 3.9</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Accuracy – Quantitative determination of Hb A1c

The levels of Hb A1c were measured twice in 40 blood samples with normal and elevated levels of Hb A1c, both by densitometry of the electrophoretic separations obtained on HYDRAGEL Hb A1c gels and by HPLC reference procedure. The measured values from both procedures were analyzed by a linear regression. The results are tabulated below (y = HYDRAGEL 15 Hb A1c).

<table>
<thead>
<tr>
<th>Mean Hb A1c (%) HPLC</th>
<th>Mean Hb A1c (%) HYDRAGEL 15 Hb A1c</th>
<th>Correlation coefficient</th>
<th>y-intercept</th>
<th>Slope</th>
<th>Range of % values (HYDRAGEL 15 Hb A1c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2</td>
<td>8.3</td>
<td>0.987</td>
<td>0.483</td>
<td>0.981</td>
<td>5.1 – 12.3</td>
</tr>
</tbody>
</table>

Linearity

The HYDRAGEL 15 Hb A1c test was determined to be linear for Hb A1c determination between 4 and 12%.

BIBLIOGRAPHY

Figure 6