

**sebia**

**HYDRAGEL 7 HEMOGLOBIN(E)**

Ref. 4106

**HYDRAGEL 15 HEMOGLOBIN(E)**

Ref. 4126

IVD

CE

R<sub>x</sub>only

2020/07

**INTENDED USE**

The HYDRAGEL 7 HEMOGLOBIN(E) and HYDRAGEL 15 HEMOGLOBIN(E) kits are designed for separation of the normal hemoglobins (A and A<sub>2</sub>) and for the detection of the major hemoglobin variants: S or D and C or E, by electrophoresis on alkaline agarose gels (pH 8.5). They are used in conjunction with the semi-automated HYDRASYS system. The resulting electrophoregrams are evaluated visually for pattern abnormalities. Densitometry can serve as an aid in the interpretation by providing relative concentrations of individual fractions. Electrophoresis on acidic gel, e.g. HYDRAGEL 7 / 15 ACID(E) HEMOGLOBIN(E), should follow to confirm the identification of hemoglobin variants, in particular, to differentiate hemoglobins S from D and E from C.

Each agarose gel is intended to run:

- 7 samples in the HYDRAGEL 7 HEMOGLOBIN(E) kit,
- 15 samples in the HYDRAGEL 15 HEMOGLOBIN(E) kit.

For *In Vitro* Diagnostic Use.

*NOTE : In this instruction sheet, the name "HYDRASYS" is used for both semi-automated HYDRASYS and HYDRASYS 2 instruments.*

**PRINCIPLE OF THE TEST<sup>1-3</sup>**

Hemoglobin is a complex molecule composed of two pairs of polypeptide chains. Each chain is linked to the heme, a tetrapyrrolic nucleus (porphyrin) which chelates an iron atom. The heme part is common to all hemoglobins and their variants. The type of hemoglobin is determined by the protein part called globin. Polypeptide chains  $\alpha$ ,  $\beta$ ,  $\delta$  and  $\gamma$  constitute the normal human hemoglobins:

- hemoglobin A ..... =  $\alpha 2 \beta 2$
- hemoglobin A<sub>2</sub> ..... =  $\alpha 2 \delta 2$
- fetal hemoglobin F ..... =  $\alpha 2 \gamma 2$

The  $\alpha$ -chain is common to these three hemoglobins.

The hemoglobin spatial structure and other molecular properties (as that of all proteins) depend on the nature and the sequence of the amino acids forming the chains. Substitution of amino acids by mutation is responsible for formation of hemoglobin variants which have different surface charge and consequently different electrophoretic mobilities, which also depend on the pH and ionic strength of the buffer.

The resulting qualitative (or structural) abnormalities are called hemoglobinopathies. Decreased synthesis of one of the hemoglobin chains leads to quantitative (or regulation) abnormalities, called thalassemias.

The assay is performed on the hemolyzate from washed red blood cells. The hemoglobins are separated by electrophoresis on alkaline gels and the fractions are visualized by staining with amidoblack. The dried gels are ready for interpretation.

**REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 7 HEMOGLOBIN(E) AND HYDRAGEL 15 HEMOGLOBIN(E) KITS**

**WARNING : See the safety data sheets.**

ITEMS	PN 4106	PN 4126
Agarose Gels (ready to use)	10 gels	10 gels
Buffered Strips (ready to use)	10 packs of 2	10 packs of 2
Ethylene glycol solution (ready to use)	1 vial, 3 mL	1 vial, 3 mL
Staining solution diluent (stock solution)	1 vial, 60 mL	1 vial, 60 mL
Amidoblack Stain (stock solution)	1 vial, 20 mL	1 vial, 20 mL
Hemolysing Solution (ready to use)	1 vial, 20 mL	1 vial, 20 mL
Applicators (ready to use)	1 pack of 10 (7 teeth)	1 pack of 10 (15 teeth)
Filter Papers	1 pack of 10	1 pack of 10

*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.*

**1. AGAROSE GELS****Preparation**

Agarose gels are ready to use. Each gel contains : agarose ; buffer solution pH 8.5  $\pm$  0.5 ; 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). (The arrow on the front of the kit box must be pointing upwards).

Avoid obvious temperature fluctuations during storage (e.g., do not store close to a window or a heat source). The gels are stable until the expiration date indicated on the kit package or the gel package labels.

DO NOT FREEZE.

Discard gel when:

- crystals or precipitate form on the gel surface or the gel texture becomes very soft (all these result from freezing the gel),
- bacterial or mold growth is indicated,
- abnormal liquid quantity 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: buffer solution pH  $9.2 \pm 0.5$  ; additives, nonhazardous at concentrations used, necessary for optimum performance.

### Use

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 (15 to 30 °C) or refrigerated (2 to 8 °C). (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. ETHYLENE GLYCOL SOLUTION

### Preparation

The ethylene glycol solution is ready to use.

### Use

To provide effective contact between the gel plastic backing and the temperature control plate of the migration module during the electrophoretic migration.

**IMPORTANT** : After each use, close immediately and tightly the ethylene glycol solution vial to avoid oxidation of the solution.

### Storage, stability and signs of deterioration

Store the ethylene glycol 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 ethylene glycol solution vial label.

## 4. STAINING SOLUTION DILUENT

### Preparation

The stock staining solution diluent must be used as described in paragraph " AMIDOBBLACK STAIN ".

It contains an acidic solution pH  $\approx 2$ .

### Use

For the preparation of the amidoblack staining solution.

### Storage, stability and signs of deterioration

Store the stock staining solution diluent 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 staining solution diluent vial labels. DO NOT FREEZE.

Do not add any sodium azide.

## 5. AMIDOBBLACK STAIN

### Preparation

The amidoblack concentrated stain is a visquous solution which may gelfy. 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.
7. Mix contents of stain cubitainer well for 5 to 10 minutes.

The staining solution is ready to use.

After dilution, the working staining solution contains : acidic solution pH  $\approx 2$  ; amidoblack ; ethylene-glycol ; additives, nonhazardous at concentrations used, necessary for optimum performance.

### Use

For staining gels with electrophoretic protein separations.

**IMPORTANT** : The staining solution is designed to stain only 10 gels. Change the solution after 10 staining steps.

### Storage, stability and signs of deterioration

Store both stock and working staining solutions at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C) 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 (2 to 8 °C). The closed container must be stored refrigerated (2 to 8 °C) immediately after each use.

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

## 6. HEMOLYSING SOLUTION

### Preparation

Hemolysing Solution is ready to use. It contains : buffer solution pH  $6.1 \pm 0.5$  ; additives, nonhazardous at the concentration used, necessary for optimum performance.

### Use

To hemolyze red blood cells.

**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.

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

**7. APPLICATORS****Use**

Precut, single use applicators for sample application.

**Storage, stability and signs of deterioration**

Store the applicators in a dry place at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C).

**8. FILTER PAPERS****Use**

Precut, single use, thin absorbent paper 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 (15 to 30 °C) or refrigerated (2 to 8 °C).

**REAGENTS REQUIRED BUT NOT SUPPLIED**

**WARNING : See the safety data sheets.**

**1. 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 an acidic solution pH  $\approx$  2.

**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 % (W/W) solution of sodium hydroxide, commercially available, into the empty waste container ( $\approx$  19 M NaOH).

**Storage, stability and signs of deterioration**

Store the stock destaining 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 destaining solution vial label. Working destaining solution is stable for one week at room temperature (15 to 30 °C) 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  $\mu$ L/dL of ProClin 300 or CLEAN PROTECT (SEBIA, PN 2059, 1 vial of 5 mL).

See the *CLEAN PROTECT* package insert for directions to use.

Working destaining solution added with ProClin or CLEAN PROTECT is stable in a closed bottle at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C) until the expiration date indicated on the kit package or destaining solution vial labels.

**2. 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 : buffer solution pH 8.7  $\pm$  0.5.

**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 (15 to 30 °C) or refrigerated (2 to 8 °C). 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.

**3. 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 (15 to 30 °C) or refrigerated (2 to 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  $\pm$  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  $\leq$  0.45  $\mu$ m) and have a conductivity lower than 3  $\mu$ S/cm, which corresponds to a resistivity higher than 0.33 M $\Omega$ .cm.*

## EQUIPMENT AND ACCESSORIES REQUIRED BUT NOT SUPPLIED

1. HYDRASYS System SEBIA: HYDRASYS 2 SCAN PN 1200, HYDRASYS 2 PN 1201, HYDRASYS 2 SCAN FOCUSING PN 1202, HYDRASYS 2 FOCUSING PN 1203, HYDRASYS PN 1210 or PN 1211 or HYDRASYS FOCUSING PN 1212.
2. Wet Storage Chamber, PN 1270, supplied with HYDRASYS.
3. Container Kit supplied with HYDRASYS.
4. Pipettes: 10  $\mu\text{L}$  and 200  $\mu\text{L}$ .
5. Densitometer / scanner capable of scanning 82 x 51 mm or 82 x 102 mm gel plates : HYRYS SEBIA, GELSCAN SEBIA, DVSE SEBIA or PHORESIS software for flat-bed scanner. Refer to manufacturer's instructions for operation and calibration procedures.
6. Gel holder for half gels, SEBIA, PN 1278.

## SAMPLES FOR ANALYSIS

### Sample collection and storage

Fresh anticoagulated blood samples are recommended for analysis. Common anticoagulants such as those containing EDTA, citrate or heparin are acceptable ; avoid those with iodoacetate. Blood must be collected according to established procedures used in clinical laboratory testing. If needed, store samples at 2 to 8 °C for up to 5 days.

### Sample preparation (standard procedure)

- **Mix the collection tube before taking the blood to prepare.**
- Centrifuge anticoagulated blood to obtain a red blood cells pellet.
- 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 10  $\mu\text{L}$ .
- Discard the excess of saline over the red blood cells pellet and vortex them before taking 10  $\mu\text{L}$  to hemolyze.
- Hemolyze 10  $\mu\text{L}$  packed red cells with 130  $\mu\text{L}$  Hemolysing Solution.
- Vortex for 10 seconds and incubate 5 minutes at room temperature (15 to 30 °C).

#### NOTES:

- To prepare hemolysate from subjects, mildly anemic (approximately 10 g/dL Hb) or severely anemic (< 7 g/dL Hb), the volume of packed RBC may be increased to 15  $\mu\text{L}$  and 20  $\mu\text{L}$ , respectively. The staining intensity will thus increase but relative concentrations of individual fractions will not change.
- The hemolysate need not be filtered or centrifuged.
- The SEBIA's hemolysing solution does not affect the unstable hemoglobin Bart's.

### Sample preparation for hemoglobin H detection

- **Mix the collection tube before taking the blood to prepare.**
- Centrifuge anticoagulated blood to obtain a red blood cells pellet.
- Discard the plasma.
- Wash the red blood cells 2 times with 10 volumes of saline ; great care must be taken when processing volumes of red blood cells smaller than 10  $\mu\text{L}$ .
- Discard the excess of saline over the red blood cells pellet and vortex them before taking 40  $\mu\text{L}$  to hemolyze.
- Hemolyze 40  $\mu\text{L}$  packed red cells with 100  $\mu\text{L}$  Hemolysing Solution.
- Vortex for 10 seconds and incubate 5 minutes at room temperature (15 to 30 °C).
- Centrifuge hemolysate at 6700 g for 5 minutes.
- The analysis is performed on the supernatant of this hemolysate ; then, follow the procedure with "7 / 15 Hb" migration program.

*NOTE : Collection tubes and centrifugation parameters for biological samples are described in the available documentation on pre-analytical phase for bio-medical analysis (data provided by the tube manufacturers, guides and recommendations on biological sample collection...). Without any indication in the instructions for use on the type of tube to use or on the centrifugation, please refer to this documentation and for the dimensions of tube to use, refer to the SEBIA document "Characteristics of tubes to use according to the instrument". The pre-analytical phase must be performed according to the state of art, the different recommendations, including those provided by the tube manufacturers, and applicable regulations.*

## 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, staining, destaining and final drying. The manual steps include handling samples and gels, and setting up the instrument for operation.

READ CAREFULLY HYDRASYS / HYDRASYS 2 INSTRUCTION MANUAL.

### I. MIGRATION SET UP

1. Switch on HYDRASYS instrument.
2. Place one applicator on a flat surface with the well numbers in the right-side-up position (Fig. 1).
  - Apply 10  $\mu\text{L}$  hemolyzed sample in each well. 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" migration program.

*NOTE: When greater separation between Hb F and Hb S is expected on HYDRAGEL 7 / 15 HEMOGLOBIN(E) gels, it is recommended to select the "7/15 Hb F-S" migration program. This program is intended only for qualitative analysis.*

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 agarose gel plate.
  - **Place its plastic side down on a tissue or filter paper to remove water droplets.**
  - 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 a too long contact with the gel to avoid its dehydration.**

  - Streak 150 µL ethylene glycol (EG) for HYDRAGEL 7 HEMOGLOBIN(E), or 300 µL for HYDRAGEL 15 HEMOGLOBIN(E), across the lower third of the frame printed on the Temperature Control Plate of the migration module.
  - **IMPORTANT:** The temperature control plate must be perfectly clean and dry before applying the ethylene glycol solution.
  - 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 slowly down onto the EG streak (Fig. 3). Ensure that no air bubbles are trapped, EG 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.
  - **Examine the applicator :** The application of a sample in the well of an applicator leads to a change in appearance of the corresponding tooth, which passes from white to translucent or more or less colored, according to the type of sample (serum, blood, urine, diluent...). Before placing an applicator on the applicator carrier, check that all teeth of the applicator are soaked with sample by examining its back side. A white tooth indicates a failure in the application or in the diffusion of the sample (defective applicator membrane, sample not applied, turbid or viscous...). In the absence of diffusion, use a new applicator and apply the samples again (treated or not according to the procedure).
  - Snap off the applicator teeth's protection frame.
  - "**7 / 15 Hb" migration program:** Place the applicator into position No. 4 on the carrier.
  - "**7 / 15 Hb F-S" migration program:** Place the applicator into position No. 3 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 340 V constant at 25 °C, controlled by Peltier effect, until 65 Vh have accumulated (for about 12 minutes, "7 / 15 Hb" migration program) or until 85 Vh have accumulated (for about 15 minutes, "7 / 15 Hb F-S" migration program).
  - The electrode carrier rises to disconnect the electrodes.
  - The temperature of the control plate rises to 50 °C for 15 minutes to dry the gel.
  - 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 25 °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. GEL PROCESSING SET-UP

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 further processing.
5. **Wipe very carefully the electrodes and the temperature control plate with a soft tissue well soaked with water.**
  - Make sure that the electrodes and the plate are well dried before re-use.
  - **IMPORTANT :** The electrodes have to be cleaned systematically after each use.
6. 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. 5).
7. 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.
8. Select "PROTEIN(E)/B1-B2/Hb" or "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).

#### III. GEL PROCESSING COMPLETION

1. Remove the gel holder from the compartment, open it and remove the dried gel.
  - *NOTE : After gel staining / destaining and before densitometry / scanning, a gel may be put through an additional wash step, if needed, to further clarify the gel background and to remove any residual stain that may appear as blue spots. Wash the gel using the "WASH ISOENZ/GEL" program.*
2. If needed, clean the back side (the plastic support side) of the dry film with a damp soft paper.

3. Scan using a densitometer / scanner by selecting the appropriate scanning program. When using HYRYS or DVSE densitometers, position the A<sub>2</sub> fraction on the 5 mm mark of the scanning plate: the background zero is made between the A<sub>2</sub> and carbonic anhydrase fractions at the lowest point.

*NOTE: To assure the most accurate and consistent results, do the following:*

- Adjust the scan length to include the entire electrophoretic pattern (≈ 30 mm).
- *If necessary, position the minima on both sides of A<sub>2</sub> fraction (if not, let the minima where they are automatically positioned).*

*It is a good practice to read the stained gels without delay. For future reference, they can be stored in a protective cover in a dry, dark place away from sources of heat and visually interpreted within at least 3 months.*

## QUALITY CONTROL

It is advised to include into each series of analysis an assayed control blood (such as Normal Hb A2 Control, SEBIA, PN 4778 or Pathological Hb A2 Control, SEBIA, PN 4779) or an assayed blood sample containing hemoglobins A, F, S and C (such as Hb AFSC Control, SEBIA, PN 4792).

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

## RESULTS

### Values

Densitometer scanning of stained electrophoregrams yields relative concentrations (percentages) of individual hemoglobin zones.

Reference values (mean ± 2 SD) on HYDRAGEL 7 / 15 HEMOGLOBIN(E) gels have been established from a healthy population of 200 adults (men and women):

Hemoglobin A	≥	96.5 %
Hemoglobin F	<	2.0 % (*)
Hemoglobin A <sub>2</sub>	≤	3.5 %

(\*) see *Interference and Limitations*

*It is recommended each laboratory establish its own reference values.*

### Interpretation

#### 1. *Qualitative abnormalities: Hemoglobinopathies*

Most hemoglobinopathies are due to substitution by mutation of a single amino acid in one of the four types of polypeptide chains. The clinical significance of such a change depends on the type of amino acid and the site involved. In clinically significant disease, either the α-chain or the β-chain is affected.

More than 200 variants of adult hemoglobin have been described. The first abnormal hemoglobins studied and the most frequently occurring have an altered net electric charge, leading to an easy detection by electrophoresis.

There are four main abnormal hemoglobins which present a particular clinical interest: S, C, E and D.

The HYDRAGEL 7 / 15 HEMOGLOBIN(E) kits are intended for the preliminary identification of hemoglobinopathies and thalassemias. Once an abnormal pattern is indicated, its identity should be confirmed by appropriate discriminatory tests (e.g., electrophoresis on acidic agarose gels).

#### **Hemoglobin S**

Hemoglobin S is the most frequent. It is due to the replacement of one glutamic acid (an acidic amino acid) of the β-chain by valine (a neutral amino acid). Its electrophoretic mobility is therefore slowed down. On alkaline buffered HYDRAGEL 7 / 15 HEMOGLOBIN(E), hemoglobin S migrates between A and A<sub>2</sub> fractions.

#### **Hemoglobin C**

One glutamic acid of the β-chain is replaced by lysine (a basic amino acid): its mobility is strongly reduced. On HYDRAGEL 7 / 15 HEMOGLOBIN(E), C, E and A<sub>2</sub> are superimposed. When this fraction is > 15 %, hemoglobins C and E must be suspected.

#### **Hemoglobin E**

One glutamic acid of the β-chain is replaced by lysine: hemoglobin E migrates exactly like hemoglobin C on HYDRAGEL 7 / 15 HEMOGLOBIN(E). Unlike hemoglobin C, it does not separate from hemoglobin A in acidic buffer [HYDRAGEL 7 / 15 ACID(E) HEMOGLOBIN(E)]. This property allows to differentiate E and C.

#### **Hemoglobin D**

One glutamic acid of the β-chain is replaced by glutamine. On HYDRAGEL 7 / 15 HEMOGLOBIN(E), this hemoglobin migrates exactly like hemoglobin S. Unlike hemoglobin S, hemoglobin D does not separate from hemoglobin A in acidic buffer [HYDRAGEL 7 / 15 ACID(E) HEMOGLOBIN(E)]; this property allows to differentiate S and D.

#### 2. *Quantitative abnormalities: Thalassemias*

Thalassemias constitute a quite heterogeneous group of genetic disorders characterized by decreased synthesis of one type of the polypeptide chains. The molecular mechanism of this decrease has not been fully described.

There are two types of thalassemia syndromes:

#### **Alpha-thalassemias**

They are characterized by the decrease of synthesis of the α-chains, consequently affecting the synthesis of all normal hemoglobins.

The excess of synthesis of the β- and γ-chains in relation to α-chains induces the formation of tetramers without any α-chain :

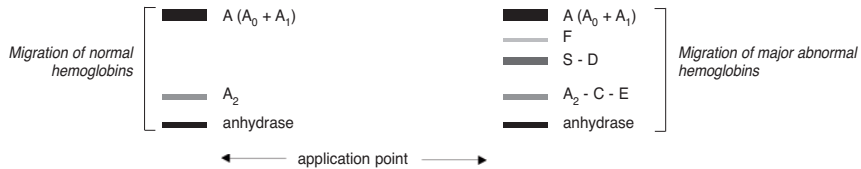
- hemoglobin Bart = γ<sub>4</sub>,
- hemoglobin H = β<sub>4</sub>.

#### **Beta-thalassemias**

They are characterized by the decrease of synthesis of the β-chains. Only hemoglobin A synthesis is affected.

Therefore hemoglobin F and hemoglobin A<sub>2</sub> percentages are increased with respect to hemoglobin A.

### 3. Migration patterns



$A_0$ : The non-glycated fraction of the normal adult hemoglobin A.

$A_1$ : The glycated fraction of the normal adult hemoglobin A.

In the above patterns, the cathode is at the bottom the anode at the top.

#### Interference and Limitations

- Do not use hemolyzed blood samples.
- When an abnormal hemoglobin is detected, use other means of identification (e.g., isoelectric focusing, globin chain electrophoresis), or consult or send sample to a specialized laboratory.
- The densitometric assay of Hb F (or of any other minor hemoglobin that migrates in the proximity of major fractions) is semi-quantitative as the values become inaccurate at below 2 % - 3 % of the total hemoglobin.
- Some homozygous "S" subjects receive a "Hydrea"<sup>®</sup> (hydroxyurea) treatment that can induce synthesis of foetal hemoglobin. The mobility of the induced hemoglobin F on HYDRAGEL 7 & 15 HEMOGLOBIN(E) has been observed in some cases slightly different from the physiological hemoglobin F.
- On samples stored more than 7 days, the smear located behind the Hb A fraction may become a concentrated fraction, do not interpret this fraction as an hemoglobin variant, e.g., H or Bart hemoglobins.

#### 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 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](http://www.sebia.com).

#### PERFORMANCE DATA

All performance data are based on a study that included SEBIA HYDRAGEL materials and instruments and comparable, commercially available agarose gel system. In addition, concordance studies were performed where the identity of hemoglobins and their values were established by other recognized methodologies.

All electrophoregrams were interpreted visually. SEBIA's HYRYS densitometer was used for all densitometric evaluations to complement the visual data as appropriate.

The results of representative examples of the performance studies are presented here. The individual values, means SD and CV are shown below. In overall, the results indicate a very good reproducibility for all the tested aspects: 2.4 % being the mean CV value with "7 / 15 Hb" migration program. The results were similar with "7 / 15 Hb F-S" migration program.

#### Reproducibility Within Run

Three blood samples were electrophoresed on HYDRAGEL 15 HEMOGLOBIN(E) gels from the same lot. Each sample was run in all the 15 tracks of a single gel. The following table shows the means, SD and CV for each individual hemoglobin component in the three samples calculated from the densitometric per cent values for each track. In addition, none of the repeats showed false positive or false negative values.

COMPONENT	MEAN (%)	SD	CV (%)
<b>Normal blood</b>			
Hb A	97.6	0.2	0.2
Hb A <sub>2</sub>	2.4	0.2	7.7
<b>Elevated Hb A<sub>2</sub></b>			
Hb A	95.3	0.2	0.2
Hb A <sub>2</sub>	4.7	0.2	5.0
<b>Elevated Hb C</b>			
Hb A	57.8	0.4	0.7
Hb C	42.2	0.4	0.9

#### Reproducibility In Between Runs

Fifteen (15) blood samples were electrophoresed on HYDRAGEL 15 HEMOGLOBIN(E) gels from a single lot. The samples analyzed included nine samples with an abnormal hemoglobin (Hb S, Hb F or Hb C) and five samples with elevated Hb A<sub>2</sub>. The means, SD and CV were calculated from the data obtained for each component in each sample. Ranges of the means, SD and CV, and the mean CV representing the pool of individual components are tabulated below. In addition, none of the repeats showed false positive or false negative values.



COMPONENT	MEAN (%)	SD	CV (%)	MEAN CV (%)
Hb A	21.6 - 98.0	0.1 - 0.6	0.1 - 2.4	0.7
Hb F	14.1 - 73.0	0.5	0.7 - 3.4	1.6
Hb C/E	20.0 - 42.4	0.3	0.7 - 1.3	1.0
Hb S/D	8.8 - 83.6	0.2 - 0.6	0.7 - 2.1	1.1
Hb A <sub>2</sub>	2.0 - 5.4	0.1 - 0.2	1.3 - 9.9	4.8

#### Accuracy - Detection of Hemoglobin Abnormalities

Sixty three (63) different blood samples were analyzed using HYDRAGEL 15 HEMOGLOBIN(E) procedure and another commercially available agarose gel system. The blood samples and their diagnostic assessment were provided by a hospital. The diagnosis was based on a routine alkaline gel and acid gel electrophoresis and/or HPLC. All abnormal hemoglobins or abnormal levels of normal hemoglobins detected with HYDRAGEL 15 HEMOGLOBIN(E) procedure were in agreement with the comparative gel system, hospital results and clinical diagnosis. There were no case observed of false positive, i.e., detection of an abnormal band or abnormal level of a normal band where no such abnormality existed.

#### Accuracy - Quantitative Determination of Hb A<sub>2</sub>

The levels of Hb A<sub>2</sub> were measured in 51 blood samples with normal and elevated levels of Hb A<sub>2</sub> both by densitometry of the electrophoretic separations obtained on HYDRAGEL 15 HEMOGLOBIN(E) gels and another commercially available agarose gel system.

The measured values from both procedures were analyzed by a linear regression statistical procedure. The results of linear regression analysis are tabulated below ( $y = \text{HYDRAGEL 15 HEMOGLOBIN(E)}$ ).

Correlation Coefficient	y-Intercept	Slope	Range of % Values (SEBIA's system)
0.981	0.103	0.906	1.7 – 5.6

#### Linearity

Two blood samples were mixed within different proportions and the dilutions were electrophoresed on HYDRAGEL 15 HEMOGLOBIN(E) gel. The test was determined to be linear within the entire range studied.

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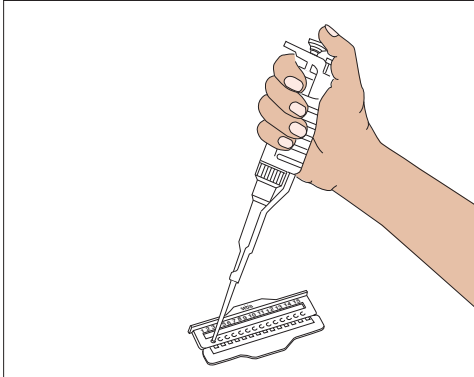
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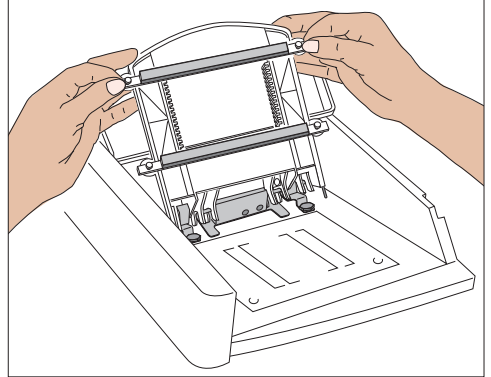
## SCHÉMAS / FIGURES

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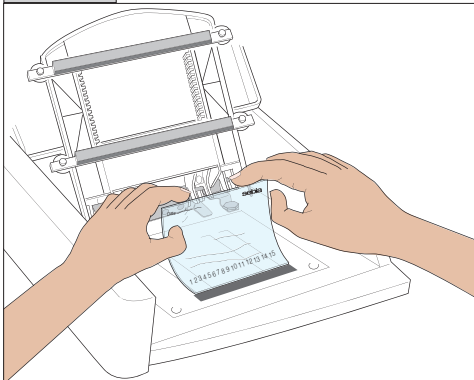
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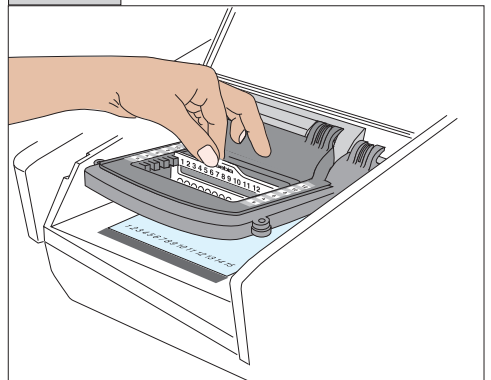
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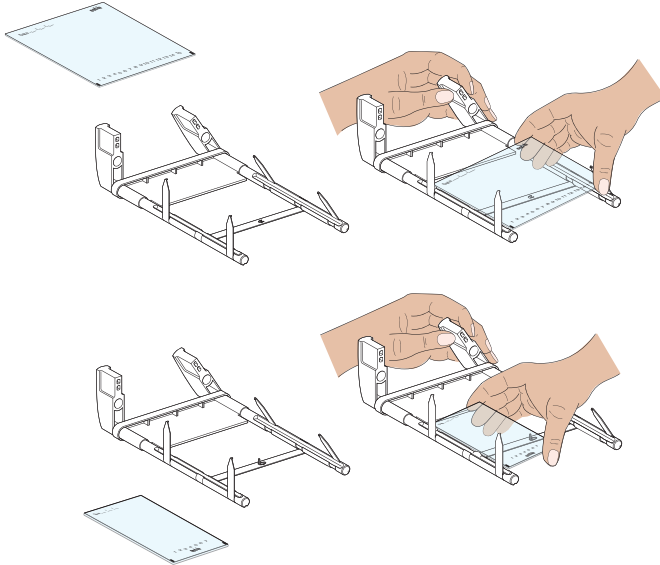


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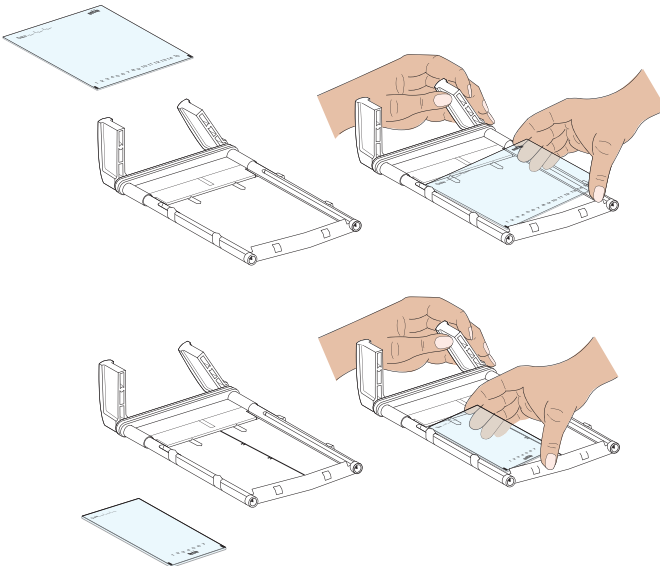
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5

## HYDRASYS



## HYDRASYS 2





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