INTENDED USE

The HYDRAGEL HEMOGLOBIN(E) K20 kit is designed for separation of the normal hemoglobins (A and A2) and for the detection of the major hemoglobin variants: S or D and C or E, by electrophoresis on alkaline agarose gels. 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 ACID(E) HEMOGLOBIN(E) K20 procedure, should follow to confirm the identification of hemoglobin variants, in particular, to differentiate hemoglobins S from D and E from C.

Each agarose gel in the HYDRAGEL HEMOGLOBIN(E) K20 kit is intended to run 7 samples.

For In Vitro Diagnostic Use.

PRINCIPLE OF THE TEST

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 α, β, δ and γ constitute the normal human hemoglobins:

- hemoglobin A = α2 β2
- hemoglobin A2 = α2 δ2
- fetal hemoglobin F = α2 γ2

The α-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 hemolyzed 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 HEMOGLOBIN(E) K20 KIT

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 3010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose Gels (ready to use)</td>
<td>10 gels</td>
</tr>
<tr>
<td>Tris-Barbital Buffer (stock solution)</td>
<td>3 vials, 75 mL each</td>
</tr>
<tr>
<td>Staining solution diluent (stock solution)</td>
<td>1 vial, 60 mL</td>
</tr>
<tr>
<td>Amidoblack Stain (stock solution)</td>
<td>1 vial, 20 mL</td>
</tr>
<tr>
<td>Destaining Solution (stock solution)</td>
<td>1 vial, 100 mL</td>
</tr>
<tr>
<td>Hemolysing solution (ready to use)</td>
<td>1 vial, 20 mL</td>
</tr>
<tr>
<td>Applicators 7 teeth (ready to use)</td>
<td>1 pack of 10</td>
</tr>
<tr>
<td>Filter Papers -Thin</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, 0.8 g/dL; alkaline buffer pH 8.5 ± 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). (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:

(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 liquid quantity is present in the gel box (as a result of buffer exudation from the gel due to improper storage conditions).

2. TRIS-BARBITAL BUFFER

Preparation

Each vial of the stock buffer solution to be diluted up to 1 liter with distilled or deionized water.

After dilution, the working solution contains: tris-barbital pH 9.2 ± 0.3; sodium azide.

WARNING: Each vial of the stock buffer contains 2.45 % barbital, 13.73 % sodium barbital and 0.13 % sodium azide. Do not ingest! If ingested, consult physician immediately! Prevent contact with acids, lead or copper, as these are known to form explosive or toxic compounds with sodium azide. Always flush with a large quantity of water when disposing.
Use
Electrophoresis buffer.

Storage, stability and signs of deterioration
Store stock buffer solution at room temperature or refrigerated. Stock solution is stable for several years, at least until the expiration date indicated on the kit package or buffer vial labels. Diluted buffer solution is stable for one year at room temperature in a closed bottle.
Discard diluted buffer if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

3. STAINING SOLUTION DILUENT
Preparation
The stock staining solution diluent must be used as described in paragraph "AMIDOBLACK STAIN".

Use
For the preparation of the amidoblack staining solution.

Storage, stability and signs of deterioration
Store the stock staining solution diluent at room temperature or refrigerated. 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.

4. AMIDOBLACK STAIN
Preparation
The amidoblack concentrated stain is a visquous 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.
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: 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 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 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.

5. DESTAINING SOLUTION
Preparation
Each vial of stock destaining solution to be diluted up to 100 liters with distilled or deionized water. It is convenient to dilute only 1 mL of the stock solution to 1 liter. 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.

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 staining vial labels.

Working destaining 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 destaining solution close to a heat source.

6. HEMOLYZING SOLUTION
Preparation
Hemolyzing Solution is ready to use. It is a buffer with additives, nonhazardous at the concentration used, necessary for optimum performance.

Use
To hemolyze red blood cells.

Storage, stability and signs of deterioration
Store Hemolyzing Solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or Hemolyzing Solution vial label.

Discard Hemolyzing 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
Store the applicators in a dry place at room temperature or refrigerated.

8. FILTER PAPERS - THIN
Use
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 or refrigerated.

REAGENTS REQUIRED BUT NOT SUPPLIED

1. 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. For longer storage periods, add sodium azide, 0.1 g/dL.

2. FIXATIVE SOLUTION (optional)
Preparation
At least 15 minutes before use, prepare a solution containing (vol. / vol.): 60 % ethanol ; 10 % acetic acid and 30 % distilled or deionized water. Mix well.
Use
To fix electrophoretic hemoglobin separations in agarose gel plates.
Storage, stability and signs of deterioration
Store fixative solution at room temperature tightly capped to prevent evaporation. Discard after 3 months. Do not fix more than 4 gels in each 150 mL of fixative solution.

EQUIPMENT AND ACCESSORIES REQUIRED BUT NOT SUPPLIED

1. Power supply: GD 61 D SEBIA, PN 1300 ; GD 251 D SEBIA, PN 1301 ; MG 300 SEBIA, PN 1302 or MG 500 SEBIA, PN 1303.
2. HYDRAGEL K20 APPLICATOR SEBIA, PN 1409, containing the HYDRAGEL K20 applicator carrier.
3. Wet Storage Chamber, PN 1270.
4. Electrophoresis chamber: K20 SEBIA, PN 1400.
5. Tanks and Gel Holders for processing of gel plates: HYDRAGEL K20 Accessory Kit SEBIA, PN 1420.
6. Pipettes: 5 µL, 10 µL and 200 µL.
7. Incubator-Dryer: IS 80 SEBIA, PN 1430.
8. Densitometer / flat-bed scanner able to scan 82 x 51 mm gel plates at 570 nm (yellow filter) : HYRYS SEBIA, DVSE SEBIA or PHORESIS software for flat-bed scanner. Refer to manufacturer’s instructions for operation and calibration procedures.

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
• 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 2 times with 10 volumes of saline; great care must be taken when processing volumes of red blood cells smaller than 10 µL.
• Discard the excess of saline over the red blood cells pellet and vortex them before taking 10 µL to hemolyze.
• Hemolyze 10 µL packed red cells with 130 µL Hemolyzing Solution.
• Vortex for 10 seconds and incubate 5 minutes at room temperature.
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 µL and 20 µL, 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 hemolyzing solution does not affect the unstable hemoglobin Bart’s.
PROCEDURE

I. MIGRATION STEP
1. Place the HYDRAGEL K20 applicator carrier on a flat surface (Fig. 1) and raise the part of the applicator carrier with the numbered notches.
2. Pool 120 µL distilled or deionized water on the lower third of the frame printed on the HYDRAGEL K20 applicator carrier.
3. Unpack the HYDRAGEL agarose gel plate.
4. 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.
5. Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame (Fig. 2).
6. Bend the gel and lower it down onto the water pool (Fig. 2). 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 the applicator carrier with the numbered notches down to the intermediate position with the switch in high position.
8. Place one applicator on a flat surface with the well numbers in the right-side-up position (Fig. 3).
9. Apply 10 µL of hemolyzed sample into the applicator wells. Load the applicator within 2 minutes.
   - Use the applicator without any delay.
   - For later use (up to 8 hours), place the applicator into the wet storage chamber with the teeth up [handle it by the plastic tooth protection frame], keep the entire chamber under refrigeration and set-up the gel plate onto the HYDRAGEL K20 applicator carrier just before use.
   See wet chamber package insert for further details.
10. Snap off the applicator teeth’s protection frame.
11. Place the sample applicator into position No. 4 on the applicator carrier.
   **IMPORTANT:** The numbers printed on the sample applicator must face the operator (Fig. 4).
12. Lower the applicator carrier with the switch so that the applicator contacts the gel surface. DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.
13. After 1 minute, turn the switch to rise up the applicator, remove the applicator and discard.
14. Put the gel into an appropriate electrophoresis chamber, according to the polarity indicated on the gel, the lower side of the gel on the cathodic side.
   When using SEBIA K20 chamber, place the HYDRAGEL on the bridge with the gel side facing down ; the gel should dip about 1 cm into the buffer on each side.
   See K20 chamber package insert for further details.
15. Plug the chamber to the power supply.

### MIGRATION CONDITIONS

<table>
<thead>
<tr>
<th>Volume of buffer per compartment</th>
<th>SEBIA K20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total buffer volume</td>
<td>150 mL</td>
</tr>
<tr>
<td>Migration time</td>
<td>300 mL</td>
</tr>
<tr>
<td>Constant voltage</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Initial current (per gel)</td>
<td>165 V</td>
</tr>
<tr>
<td></td>
<td>7 ± 2 mA</td>
</tr>
</tbody>
</table>

16. After migration, unplug the chamber and remove the gel plate.

II. FIXATION

Process gels according to one of the following procedures.

**Hot air fixation (recommended only with SEBIA IS 80 Incubator-Dryer):**
Dry the gel completely in the incubator-dryer at 80 °C (for 10 minutes minimum).

**Fixation with fixative solution:**
1. Place the gel into a gel holder (supplied with SEBIA HYDRAGEL K20 Accessory Kit) for further processing.
2. Fill one tank (supplied with SEBIA HYDRAGEL K20 Accessory Kit) with 150 mL of fixative solution.
3. Immerse the gel in the fixative solution for 15 minutes.
4. Remove the gel and dry it with hot 80 °C air flow in incubator-dryer IS 80.
   **IMPORTANT:** The gel must be perfectly dry.

III. STAINING - DESTAINING

1. Immerse the dried and cooled gel in the staining solution for 5 minutes.
2. Destain in three successive baths of destaining solution until the background is completely colorless and clear.
3. Soak up excess liquid on the gel surface with a tissue paper and dry the gel with hot 80 °C air. If needed, clean the back side (the plastic support side) of the dry film with a wet tissue paper.

IV. SCANNING

Scan using a densitometer / scanner at 570 nm or with a yellow filter. When using HYRYS or DVSE, densitometers, position the A2 fraction on the 5 mm mark of the scanning plate: the background zero is made between the A2 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).
- Make sure the minima on both sides of A2 fraction are positioned at the very feet of the A2 peak.

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.
**RESULTS**

**Quality Control**

It is advised to include an assayed control blood or assayed blood sample containing hemoglobins A, F, C and S into each run of samples.

**Values**

Densitometer scanning of stained electrophoregrams yields relative concentrations (percentages) of individual hemoglobin zones. Normal values (mean ± 2 SD) using HYDRAGEL HEMOGLOBIN(E) K20 procedure have been established from a healthy population of 200 adults (men and women):

- Hemoglobin A ≥ 96.5 %
- Hemoglobin F < 2.0 % (*)
- Hemoglobin A₂ ≤ 3.5 %

(*') see Interference and Limitations

It is recommended each laboratory establishes its own normal 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 (even γ chains).

   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 HEMOGLOBIN(E) K20 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 gels, HYDRAGEL HEMOGLOBIN(E) K20, hemoglobin S migrates between A and A₂ fractions.

   - **Hemoglobin C**
     
     One glutamic acid of the β-chain is replaced by lysine (a basic amino acid): its mobility is strongly reduced. With HYDRAGEL HEMOGLOBIN(E) K20 procedure, C, E and A₂ 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 using HYDRAGEL HEMOGLOBIN(E) K20 procedure. Unlike hemoglobin C, it does not separate from hemoglobin A in acidic buffer [HYDRAGEL ACID(E) HEMOGLOBIN(E) K20]. This property allows to differentiate E and C.

   - **Hemoglobin D**
     
     One glutamic acid of the β-chain is replaced by glutamine. With HYDRAGEL HEMOGLOBIN(E) K20 procedure, this hemoglobin migrates exactly like hemoglobin S. Hemoglobin D does not separate from hemoglobin A in acidic buffer [HYDRAGEL ACID(E) HEMOGLOBIN(E) K20]; 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 or several types 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 chains induces the formation of tetrameres without any α-chain:
     
     - hemoglobin Bart = γ₄
     - hemoglobin H = β₄

   - **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 A₂ percentages are increased with respect to hemoglobin A.

3. **Migration patterns**

   ![Migration patterns diagram](image)

   A₀ : The non-glycated fraction of the normal adult hemoglobin A.
   A₁ : 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, which behaves differently than the major hemoglobin variants S, C, D and E, 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® (hydroxyurea)" treatment that can induce synthesis of foetal hemoglobin. The mobility of the induced hemoglobin F on HYDRAGEL 7 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 informations on waste products elimination are available from the Technical Service of the supplier.

PERFORMANCE DATA

All performance data are based on a study that included SEBIA HYDRAGEL materials and instruments compared to a 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. The results of representative examples of the performance studies are presented here.

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

Reproducibility Within Run

Three blood samples were electrophoresed using HYDRAGEL HEMOGLOBIN(E) K20 procedure on gels from the same lot. Each sample was run in all the 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.

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>MEAN</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb A</td>
<td>97.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Hb A₂</td>
<td>2.6</td>
<td>0.1</td>
<td>5.3</td>
</tr>
<tr>
<td>Elevated Hb A₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb A</td>
<td>95.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Hb A₂</td>
<td>4.8</td>
<td>0.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Elevated Hb C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb A</td>
<td>56.7</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Hb C</td>
<td>43.3</td>
<td>0.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Reproducibility In Between Runs

Blood samples were electrophoresed using HYDRAGEL HEMOGLOBIN(E) K20 procedure on gels from a single lot. The samples analyzed included seven samples with an abnormal hemoglobin (Hb S, Hb F or Hb C), two samples with elevated Hb A₂ and the rest were normal blood samples. 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.

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>MEAN</th>
<th>SD</th>
<th>CV (%)</th>
<th>MEAN CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A</td>
<td>18.0 – 97.8</td>
<td>0.1 – 1.0</td>
<td>0.1 – 4.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Hb F</td>
<td>13.3 – 76.3</td>
<td>0.5 – 0.6</td>
<td>0.7 – 3.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Hb C</td>
<td>21.5 – 43.5</td>
<td>0.2 – 0.3</td>
<td>0.6 – 1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Hb S/D</td>
<td>9.7 – 84.1</td>
<td>0.1 – 0.6</td>
<td>0.4 – 2.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Hb A₂</td>
<td>2.2 – 5.7</td>
<td>0.1 – 0.2</td>
<td>2.3 – 7.6</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Accuracy - Detection of Hemoglobin Abnormalities

Sixty three different blood samples were analyzed using HYDRAGEL HEMOGLOBIN(E) K20 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 HEMOGLOBIN(E) K20 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 A₂

The levels of Hb A₂ were measured in 51 blood samples with normal and elevated levels of Hb A₂ both by densitometry of the electrophoretic separations obtained using HYDRAGEL HEMOGLOBIN(E) K20 procedure. The measured values from both procedures were analyzed by a linear regression statistical procedure. The results of linear regression analysis are tabulated below (y = HYDRAGEL).

<table>
<thead>
<tr>
<th>Correlation Coefficient</th>
<th>y-Intercept</th>
<th>Slope</th>
<th>Range of % Values (SEBIA's system)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.973</td>
<td>-0.344</td>
<td>0.963</td>
<td>1.5 – 5.7</td>
</tr>
</tbody>
</table>
BIBLIOGRAPHY
