

# QuickGel® Alkaline Hemoglobin Procedure

Helena  Laboratories

Cat. No. 3429, 3529, 3529T

The QuickGel Alkaline Hemoglobin electrophoresis procedure is intended for the qualitative and semi-quantitative determination of hemoglobins using agarose electrophoresis in alkaline buffer on the SPIFE 2000 or SPIFE 3000 or the QuickGel system. The system is used as a screening method for in-vitro diagnostic use.

## SUMMARY

Hemoglobins (Hb) are a group of proteins whose chief functions are to transport oxygen from the lungs to the tissues and carbon dioxide in the reverse direction. They are composed of polypeptide chains, called globin, and iron protoporphyrin heme groups. A specific sequence of amino acids constitutes each of four polypeptide chains. Each normal hemoglobin molecule contains one pair of alpha and one pair of non-alpha chains. In normal adult hemoglobin (HbA), the non-alpha chains are called beta. The non-alpha chains of fetal hemoglobin are called gamma. A minor (3%) hemoglobin fraction called HbA<sub>2</sub> contains alpha and delta chains. Two other chains are formed in the embryo.

The major hemoglobin in the erythrocytes of the normal adult is HbA and there are small amounts of HbA<sub>2</sub> and HbF. In addition, over 400 mutant hemoglobins are now known, some of which may cause serious clinical effects, especially in the homozygous state or in combination with another abnormal hemoglobin. Wintrobe<sup>1</sup> divides the abnormalities of hemoglobin synthesis into three groups.

- (1) Production of an abnormal protein molecule (e.g. sickle cell anemia)
- (2) Reduction in the amount of normal protein synthesis (e.g. thalassemia)
- (3) Developmental anomalies (e.g. hereditary persistence of fetal hemoglobin (HPFH))

The two mutant hemoglobins most commonly seen in the United States are HbS and HbC. Hb Lepore, HbE, HbG-Philadelphia, HbD-Los Angeles, and HbO-Arab may be seen less frequently.<sup>2</sup>

Electrophoresis is generally considered the best method for separating and identifying hemoglobinopathies.<sup>3</sup> The protocol for hemoglobin electrophoresis involves step-wise use of two systems.<sup>4,9</sup> Initial electrophoresis is performed in alkaline buffers. Cellulose acetate used to be the major support medium used, however agarose also yields rapid separation of HbA, F, S, and C and many other mutants with minimal preparation time. However, because of the electrophoretic similarity of many structurally different hemoglobins, the evaluation must be supplemented by citrate agar electrophoresis which measures a property other than electrical charge.

This method is based on the complex interactions of the hemoglobin with an alkaline electrophoretic buffer and the agarose support. The QuickGel Alkaline Hemoglobin procedure is a simple procedure requiring minute quantities of hemolysate to provide a screening method for the presence of abnormal hemoglobins, such as HbS, HbC and HbF.

## PRINCIPLE

Very small samples of hemolysates prepared from packed cells are applied to the QuickGel Alkaline Hemoglobin gel. The hemoglobins in the sample are separated by electrophoresis using an alkaline buffer and are stained with Acid Blue Stain. The patterns are scanned on a densitometer, and the relative percent of each band is determined.

## REAGENTS

### 1. QuickGel Alkaline Hemoglobin Gel

**Ingredients:** Each gel contains agarose in tris, glycine buffer with 0.05% EDTA and sodium azide as a preservative.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.** The gel contains barbital which, in sufficient quantity, can be toxic. To prevent the formation of toxic vapors, sodium azide should not be mixed with acidic solutions. When discarding reagents containing sodium azide, always flush sink with copious quantities of water. This will prevent the formation of metallic azides which, when highly concentrated in metal, are potentially explosive. In addition to purging with water, plumbing should occasionally be decontaminated with 10% NaOH.

**Preparation for Use:** The gels are ready for use as packaged.

**Storage and Stability:** The gels should be stored horizontally at room temperature (15 to 30°C) and are stable until the expiration date indicated on the package. The gels must be stored in the protective packaging in which they are shipped. **DO NOT REFRIGERATE OR FREEZE THE GELS.**

**Signs of Deterioration:** Any of the following conditions may indicate deterioration of the gel: (1) Crystalline appearance indicating the agarose has been frozen, (2) cracking and peeling indicating drying of the agarose, (3) bacterial growth indicating contamination, (4) thinning of the gel blocks.

### 2. Acid Blue Stain

**Ingredients:** When dissolved as directed, the stain contains 0.5% (w/v) acid blue stain.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.**

**Preparation for Use:** Dissolve the dry stain (entire contents of vial) in 1 L of 5% acetic acid. Mix thoroughly for 30 minutes.

**Storage and Stability:** The dry stain should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. The diluted stain is stable six months when stored at 15 to 30°C.

**Signs of Deterioration:** The diluted stain should be a homogeneous mixture free of precipitate. Discard if precipitate forms.

### 3. Hemolysate Reagent

**Ingredients:** The reagent is an aqueous solution of 0.005 M EDTA, 0.175% saponin and 0.07% potassium cyanide.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT PIPETTE BY MOUTH.** The reagent contains potassium cyanide.

**Preparation for Use:** The reagent is ready for use as packaged.

**Storage and Stability:** The reagent should be stored at room temperature (15 to 30°C) and is stable until the expiration date indicated on the vial.

**Signs of Deterioration:** The reagent should be a clear, yellow solution.

### 4. Citric Acid Destain

**Ingredients:** After dissolution, the destain contains 0.3% (w/v) citric acid.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST - IRRITANT.**

**Preparation for Use:** Pour 11 L of deionized water into the Destain vat. Add the entire package of Destain. Mix well until completely dissolved.

**Storage and Stability:** Store the Destain at 15 to 30°C. It is stable until the expiration date on the package.

**Signs of Deterioration:** Discard if solution becomes cloudy.

## INSTRUMENT

A SPIFE 2000 or 3000 analyzer or the QuickGel Chamber must be used to electrophorese, stain, destain, and then dry the gels. The gels may be scanned on a separate densitometer or the QuickScan 2000 (Cat. No. 1660). Refer to the appropriate Operator's Manual for detailed instructions.

## SPECIMEN COLLECTION AND HANDLING

**Specimen:** Whole blood collected in EDTA tubes is the specimen of choice.

**Specimen Storage:** If storage is necessary, whole blood and packed cells may be stored up to 1 week at 2 to 8°C.

**Specimen Preparation:** Washed packed cell hemolysates must be prepared for each patient specimen.

a) Whole Blood sample

1. Centrifuge anticoagulated blood for 10 minutes to separate cells from plasma.
2. Remove plasma.
3. Wash packed cells 3 times by resuspending in 5 to 10 volumes of normal saline solution (0.85% NaCl), centrifuging and aspirating supernatant as before.

- After washing the samples, prepare the hemolysates by mixing 10  $\mu$ L sample to 100  $\mu$ L Hemolysate Reagent. Vortex or shake vigorously for 15 seconds.

b) Control

- AA<sub>2</sub> (Cat. No. 5328) no dilution is necessary
- AFSC (Cat. No. 5331) 1:2 (1 part control + 1 part Hemolysate Reagent)

**PROCEDURE FOR SPIFE 2000/3000**

**Materials provided:** The following materials needed for the procedure are contained in the QuickGel Alkaline Hemoglobin Kit (Cat. No. 3429). Individual items are not available.

- QuickGel Alkaline Hemoglobin Gels (10)
- Acid Blue Stain (1 vial)
- Hemolysate Reagent (25 mL)
- Citric Acid Destain (1 pkg)
- QuickGel Blotter C (10)
- QuickGel Blotter X (20)
- Applicator Blade Assembly (10)

**Materials available but not contained in the kit:**

ITEM	CAT. NO.
SPIFE 3000 Analyzer	1088
SPIFE 2000 Analyzer	1130
QuickScan 2000	1660
AFSC Hemo Control	5331
AA <sub>2</sub> Hemo Control	5328
REP Prep	3100
SPIFE QuickGel Electrode	1111
SPIFE QuickGel Holder	3358
SPIFE Gel Block Remover	1115
SPIFE QuickGel Chamber Alignment Guide	86541003
Applicator Blade Weights	3387
Disposable Sample Cups	3369
QuickGel Dispo Cup Tray	3353
Chamber Cover	8JP34012

**Materials needed but not provided:**

- 5% Acetic acid
- 0.85% NaCl

**STEP BY STEP METHOD**

**I. Chamber Preparation**

- The SPIFE QuickGel Chamber Alignment Guide must be used to mark the location for gel placement on the chamber floor if not marked previously. It is recommended that the markings be placed directly on the copper floor under the contact sheet.
- Remove the contact sheet & clean the chamber floor according to instructions in the Operator's Manual.
- Place the round hole in the guide over the left chamber pin and the obround hole over the right pin.
- Using an indelible marker, outline the rectangular open area onto the copper floor. Allow marking to dry, and apply another contact sheet.

**II. Sample Preparation**

- Prepare hemolysates of patient specimens and controls as instructed in the "Specimen Preparation" section.

- Remove one disposable Applicator Blade Assembly from the packaging. Remove the protective guard from the blade by gently bending the protective piece back and forth until it breaks free.



- Place the Applicator Blade into the vertical slot numbered 6 in the Applicator Assembly.

**Please note that the blade assembly will only fit into the slots in the Applicator Assembly one way; do not try to force the Applicator Blade into the slots.**

- Place an Applicator Weight on top of the Applicator Blade.
- Slide the Disposable Sample Cup strip into the row numbered 1 to 20 of the Cup Tray.
- Pipette 17  $\mu$ L of patient or control hemolysate into the Disposable Cups numbered 1 to 5 and 6 to 10. Cover the tray until ready for use.

**III. Gel Preparation**

- Carefully open one end of the pouch and remove one gel from

the protective packaging. Reseal the pouch with tape to prevent drying of the gel. Remove the gel from the plastic mold and discard the mold.

- Use a QuickGel Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
- Dispense about 1 mL of REP Prep onto the left side of the marked area.
- Place the gel over the REP Prep inside the rectangle on the chamber floor. Hold the gel so that the samples numbered 1 to 10 are turned to the left side of the chamber. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side. Use lint-free tissue to wipe around the edges of the gel backing to remove excess REP Prep. Make sure the gel remains in place and that no bubbles remain under the gel.
- Clean the QuickGel electrodes with deionized water before and after each use. Wipe the electrodes with lint-free tissue.
- Place a QuickGel electrode on the outside ledge of each gel block inside the magnetic posts. Improper contact between the electrodes and the gel block can result in skewed patterns. Close the chamber lid.
- Press the **TEST SELECT/CONTINUE** button on the Electrophoresis and Stainer sides of the instrument until **QG-ALKALINE HEMOGLOBIN or ALKALINE HEMOGLOBIN** option appears on the display.

**IV. Electrophoresis/Staining**

Using the instructions provided in the appropriate Operator's Manual, set up parameters as follows for the SPIFE 2000 or the SPIFE 3000.

**NOTE: A "Dry 1" time of 15 minutes and a "Dry 2" time of 7 minutes are recommended. However, due to variations in environmental conditions, the following ranges are acceptable.**

\* Dry 1 = 12 to 16 minutes

\*\* Dry 2 = 5 to 8 minutes

**A. SPIFE 3000**

**Electrophoresis Unit**

- No Prompt  
Load Sample 1      00:30    20°C    SPD4
- No Prompt  
Apply Sample 1    00:30    20°C    SPD4    LOC 1
- To Continue (continue)  
Electrophoresis 1    15:00    18°C    550V    35 mA
- No Prompt  
END OF TEST

**Stainer Unit**

- No Prompt  
Stain 1                    4:00    REC=OFF    VALVE=3
- No Prompt  
Destain 1                0:30    REC=ON    VALVE=2
- No Prompt  
Dry 1                      \*15:00    70°C
- No Prompt  
Destain 2                1:30    REC=ON    VALVE=2
- No Prompt  
Destain 3                1:30    REC=ON    VALVE=2
- No Prompt  
Dry 2                      \*\*7:00    70°C
- No Prompt  
END OF TEST

**B. SPIFE 2000**

**Electrophoresis Unit**

- No Prompt  
Load Sample 1      00:30    20°C    SPD.=4
- No Prompt  
Apply Sample 1    00:30    20°C    SPD.=4
- To Continue (continue)  
Electrophoresis 1    15:00    18°C    550V
- No Prompt  
END OF TEST

Stainer Unit			
1) No Prompt Stain 1	4:00	Cir=OFF	VALVE=3
2) No Prompt Destain 1	0:30	Cir=ON	VALVE=2
3) No Prompt Dry 1	*15:00	70°C	
4) No Prompt Destain 2	1:30	Cir=ON	VALVE=2
5) No Prompt Destain 3	1:30	Cir=ON	VALVE=2
6) No Prompt Dry 2	**7:00	70°C	
7) No Prompt			
END OF TEST			

- Open the chamber lid. Place the Cup Tray with samples on the SPIFE 2000/3000. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
- With appropriate test name on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. The SPIFE 2000/3000 will apply the samples, electrophoresis and beep when completed.
- Open the chamber lid. Remove two QuickGel Blotter X's from the package. Place a blotter horizontally across the top and bottom of the gel backing so that the blotters overlap the edge of the backing, but do not touch the agarose gel. The blotters will run lengthwise along the gel and will be centered between the electrodes.
- Insert a Chamber Cover into the grooves of the chamber and close the chamber lid. Press **TEST SELECT/CONTINUE** to start electrophoresis.
- At the end of the electrophoresis, the instrument will beep. Open the Chamber Lid, remove the Chamber Cover, electrodes and blotters. Dispose of the blade and cups as biohazardous waste.
- With the gel still in the chamber, use a Gel Block Remover or straight edge to completely remove and discard the gel blocks. Then remove the gel from the chamber.

#### V. Visualization

- Remove the SPIFE QuickGel Holder from the stainer chamber. While holding the gel agarose side down, slide one side of the gel backing under one of the metal bars. Bend the gel backing so that the gel is bowed, and slip the other side under the other metal bar. The two small notches in the backing must fit over the small pins to secure the gel to the holder.
- Place the Gel Holder with the attached gel facing backwards into the stainer chamber.
- Press the **TEST SELECT/CONTINUE** button until the test name appears on the display.
- Press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. The instrument will stain, destain, and dry the gel.
- When the process is complete, the instrument will beep. Carefully remove the SPIFE QuickGel Holder from the stainer because the metal piece on the holder will be hot. Take the gel off of the holder and replace the holder. Refer to **Evaluation of the Hemoglobin Bands**.

#### PROCEDURE FOR QuickGel® CHAMBER

The following instructions are for using the QuickGel Chamber (Cat. No. 1284) for electrophoresis.

**Materials Provided:** The following materials needed for the procedure are contained in the QuickGel Alkaline Hemoglobin Kit (Cat. No. 3529 and 3529T). Individual items are not available.

##### Cat. No. 3529

QuickGel Alkaline Hemoglobin Gels (10)  
Acid Blue Stain (1 vial)  
QuickGel Blotter C (10)  
QuickGel Blotter X (20)  
Citric Acid Destain (1 pkg)

##### Cat. No. 3529T

QuickGel Alkaline Hemoglobin Gels (10)  
Acid Blue Stain (1 vial)  
QuickGel Blotter C (10)  
QuickGel Blotter A (10)  
QuickGel Blotter X (20)

Hemolysate Reagent (25 mL)  
QuickGel Applicator Blades (10)  
QuickGel Dispo Sample Cups (10)  
Citric Acid Destain (1 pkg)  
Hemolysate Reagent (25 mL)  
QuickGel Sample Templates (10)

#### Materials available but not contained in the kit:

ITEM	CAT. NO.
QuickGel Chamber	1284
QuickGel Applicator	1265
QuickGel Applicator Base	1266
QuickGel Applicator Weights	1267
QuickGel Dispo Cup Tray	1268
QuickGel Dispo Sample Cups	1269
QuickGel Applicator Blades	1270
QuickGel Gel Block Remover	1262
QuickGel Applicator Kit	1274

(Includes Applicator, Applicator Base, Weights, and Cup Tray)

#### Materials needed but not provided:

Power Supply capable of providing at least 550 Volts  
5% Acetic acid  
0.85% NaCl

#### STEP BY STEP METHOD

**NOTE:** The use of templates for sample application is offered as an option instead of the Applicator. Instructions are provided for both methods in Sections II and IV.

##### I. Chamber Preparation

- The QuickGel Chamber must be plugged into a power supply. Set a timer for \*14:30 minutes and the power at 550 Volts. \*An electrophoresis time of 14 to 15 minutes is acceptable.
- Snap the Electrophoresis Lid into place on the chamber.
- Ensure that the chamber floor is cool (room temperature) before starting the test.

##### II. Sample Preparation

###### A. Applicator Blade Method

- Prepare hemolysates of patient specimens and controls as instructed in the "Specimen Preparation" of the **SPECIMEN COLLECTION AND HANDLING** section.
- Slide the QuickGel Disposable Sample Cups into Row A of the Dispo Cup Tray. Pipette 17 µL of patient or control hemolysate into the Sample Cups and cover tray until ready to use. When ready, place the Dispo Cup Tray into the Applicator Base. Proceed to Section III.

###### B. Sample Template Method

- Prepare hemolysates of patient specimens and controls as instructed in the "Specimen Preparation" of the **SPECIMEN COLLECTION AND HANDLING** section with the following changes:
  - Whole Blood Sample  
After washing the samples, prepare the hemolysates by mixing 10 µL sample to 150 µL Hemolysate Reagent. Vortex or shake vigorously for 15 seconds.
  - Control  
AA<sub>2</sub> (Cat. No. 5328) no dilution is necessary  
AFSC (Cat. No. 5331) 1:4 (1 part control + 3 parts Hemolysate Reagent)

##### III. Gel Preparation

- Carefully cut open one end of the gel pouch. Remove one gel from the protective packaging. Fold and tape the open end of the pouch to prevent drying of the remaining gel. Remove the gel to be used from the plastic mold and discard the mold.
- Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
- Hold the gel so that the samples numbered 1 to 10 are turned to the left side of the chamber. Place the left notch of the gel so that it fits the left pin of the chamber floor and gently roll the gel to the right side fitting the right notch to the right pin of the chamber floor. Use a lint free tissue to wipe around the edges of the gel backing to remove any excess REP Prep. Make sure that no bubbles remain under the gel.
- Using a QuickGel Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.

#### IV. Sample Application

##### A. Applicator Blade Method

1. Remove one QuickGel Applicator Blade from the packaging.
2. Place an Applicator Weight on top of the Applicator Blade. Place the blade into the vertical slot A of the Applicator.
3. While holding the white Applicator Knob up, place the Applicator into the designated slot on the Applicator Base aligning the small red dots on the Applicator with those on the Base.
4. Then slowly lower the Applicator Knob allowing the blade to enter the sample cups and immediately start a timer for 30 seconds.
5. After 30 seconds, lift the Applicator Knob and immediately place the Applicator into the slot on the chamber floor, aligning the red dots.
6. Slowly lower the Applicator Knob to apply sample to the gel. Set a timer for 30 seconds.
7. After the 30 seconds application, lift the Applicator Knob and remove the Applicator from the chamber.
8. Place two Blotter X's horizontally along the top and bottom sides of the gel backing. They should be positioned along the edges (not touching the gel) so that, when the lid closes, the Blotter X's do not interfere with the electrodes.
9. Close the lid, press the power switch to turn on the chamber and power supply. Proceed to Section V.

##### B. Sample Template Method

1. Remove one QuickGel Sample Template from the package. Hold the template so that the small hole in the corner is toward the front right side of the chamber.
2. Carefully place the template on the gel aligning the template slits with the marks on each side of the gel backing. The center hole in the template should align with the indentation in the center of the gel.
3. Apply slight fingertip pressure to the template making sure there are no bubbles under it. **NOTE:** If wearing rubber gloves to perform this step, place a QuickGel Blotter A over the template and then apply fingertip pressure to the template. Remove the blotter. Powder from the gloves can produce gel artifacts.
4. Pipette 3  $\mu$ L of each hemolysate onto the template slits. Wait 2 minutes after the last sample application to allow proper sample absorption.
5. Use the QuickGel Blotter A to gently blot the excess sample from the template. Then carefully remove the templates
6. Place two Blotter X's horizontally along the top and bottom sides of the gel backing. They should be positioned along the edges (not touching the gel) so that, when the lid closes, the Blotter X's do not interfere with the electrodes.
7. Close the lid, press the power switch to turn on the chamber and start the power supply.

#### V. Electrophoresis and Staining

1. Electrophorese the gel for \*14:30 minutes at 550 Volts.
2. Turn off the Power Supply and the QuickGel Chamber.
3. Remove the Blotter X's. Using the QuickGel Gel Block Remover, remove the two gel blocks from the gel. Again, use a lint free tissue to wipe around the edges of the gel backing to remove any excess moisture. Remove the gel from the chamber.
4. Replace the Electrophoresis Lid with the Drying Lid. Clean the two electrodes on the Electrophoresis Lid with deionized water after each use. Wipe with a lint-free tissue.
5. Fill a Staining Dish with prepared stain. Fill another container with Destain solution.
6. Place the gel into the stain for 4 minutes. Remove the gel from the stain and allow it to drain on a blotter.
7. Then place the gel into Destain Solution for 30 seconds, remove it and allow it to drain on a blotter.
8. Carefully place the gel in the chamber and close the Drying Lid.
9. Turn on only the QuickGel Chamber. Dry the gel for the 25 minutes or until dry. After drying, turn the Chamber off and remove the gel.

10. Destain the gel in two consecutive washes of Destain solution. Use a gentle alternately rocking and swirling technique. Allow the gel to remain in each wash for 1:30 minutes. The gel background should be completely clear. Tap the gel to remove the excess destain solution.
11. Ensure that the chamber floor is clean. Replace the gel onto the QuickGel Chamber floor. Close the Drying Lid, turn on the QuickGel Chamber and dry for 10 minutes or until dry. When drying is complete, turn off QuickGel Chamber and remove the gel.

#### Evaluation of the Hemoglobin Bands

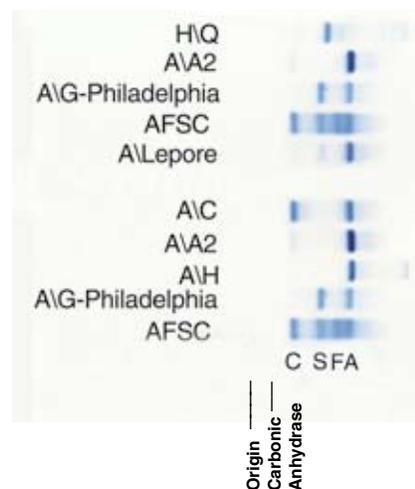
1. **Qualitative evaluation:** The hemoglobin gels may be inspected visually for the presence of abnormal hemoglobin bands. The Helena Hemo Controls provide a marker for band identification.
2. **Quantitative evaluation:** Determine the relative percent of each hemoglobin band by scanning the dried gels in the QuickScan using Acid Blue filter.

**Stability of End Product:** The dried gels are stable for an indefinite period of time.

**Quality Control:** Two controls for hemoglobin electrophoresis are available from Helena Laboratories: AA<sub>2</sub> Hemo Control (Cat. No. 5328) and AFSC Hemo Control (Cat. No. 5331). The controls should be used as markers for the location of particular hemoglobin bands. They may be quantitated for verification of the accuracy of the procedure (see "Limitations" section). Refer to the package insert provided with the controls for assay values and migration patterns. Use at least one of these controls on each gel run.

#### RESULTS

Figure 1 illustrates the electrophoretic mobility of bands on the QuickGel Alkaline Hemoglobin Gel.



#### LIMITATIONS

Some abnormal hemoglobins have similar electrophoretic mobilities and must be differentiated by other methodologies.

##### Further testing required:

1. Citrate agar electrophoresis may be a necessary follow-up test for confirmation of abnormal hemoglobins detected.
2. Globin chain analysis (both acid and alkaline) and structural studies may be necessary in order to positively identify some of the more rare hemoglobins.
3. Anion exchange column chromatography is a more accurate method for quantitating HbA<sub>2</sub>. Helena Laboratories' Sickie-Thal Quik Column® Method (Cat. No. 5334) for quantitation of HbA<sub>2</sub> in the presence of HbS, or the Helena Beta-Thal HbA<sub>2</sub> Quik Column® Procedure (Cat. No. 5341) are recommended. HbA<sub>2</sub> quantitation is one of the most important diagnostic tests in the diagnosis of thalassemia trait.
4. Low levels of HbF (1-10%) may be accurately quantitated by radial immunodiffusion using the Helena HbF-QUIPlate Procedure (Cat. No. 9325).

## REFERENCE VALUES

At birth, the majority of hemoglobin in the erythrocytes of the normal individual is fetal hemoglobin, HbF. Some of the major adult hemoglobin, HbA, and a small amount of HbA<sub>2</sub>, are also present. At the end of the first year of life and through adulthood, the major hemoglobin present is HbA with up to 3.7% HbA<sub>2</sub> and less than 2% HbF<sup>3</sup>.

A study of forty-eight (48) normal adult specimens was done using the QuickGel system on the SPIFE. The data was as follows:

HbA - 96.5% - 98.1%

HbA<sub>2</sub> - 1.9% - 3.5%

These values should only serve as guidelines. Each laboratory should establish its own range.

## INTERPRETATION OF RESULTS

Most hemoglobin variants cause no discernible clinical symptoms, so are of interest primarily to research scientists. Variants are clinically important when their presence leads to sickling disorders, thalassemia syndromes, life long cyanosis, hemolytic anemias or erythrocytosis or if the heterozygote is of sufficient prevalence to warrant genetic counseling. The combinations of HbSS, HbSD-Los Angeles, and HbSO-Arab lead to serious sickling disorders.<sup>2</sup> Several variants including HbH, E-Fort Worth and Lepore cause a thalassemic blood picture.<sup>2</sup>

The two variant hemoglobins of greatest importance in the U.S., in terms of frequency and pathology, are HbS and HbC<sup>2</sup>. Sickle cell anemia (HbSS) is a cruel and lethal disease. It first manifests itself at about 5-6 months of age. The clinical course presents agonizing episodes of pain and temperature elevations with anemia, listlessness, lethargy, and infarct in virtually all organs of the body. The individual with homozygous HbCC suffers mild hemolytic anemia which is attributed to the precipitation or crystallization of HbC within the erythrocytes. Cases of HbSC disease are characterized by hemolytic anemia that is milder than sickle cell anemia.

The thalassemias are a group of hemoglobin disorders characterized by hypochromia and microcytosis due to the diminished synthesis of one globin chain (the  $\alpha$  or  $\beta$ ) while synthesis of the other chain proceeds normally.<sup>10,11</sup> This unbalanced synthesis results in unstable globin chains. These precipitate within the red cell, forming inclusion bodies that shorten the life span of the cell. In  $\alpha$ -thalassemias, the  $\alpha$  chains are diminished or absent, and in the  $\beta$ -thalassemia, the  $\beta$  chains are affected. Another quantitative disorder of hemoglobin synthesis, hereditary persistent fetal hemoglobin (HPFH), represents a genetic failure of the mechanisms that turn off gamma chain synthesis at about four months after birth which results in a continued high percentage of HbF. It is a more benign condition than the true thalassemias and persons homozygous for HPFH have normal development, are asymptomatic and have no anemia.<sup>11</sup>

The most common hemoglobin abnormalities:

### Sickle Cell Trait

This is a heterozygous state showing HbA and HbS and a normal amount of HbA<sub>2</sub> on cellulose acetate. Results on citrate agar show hemoglobins in the HbA and HbS migratory positions (zones).

### Sickle Cell Anemia

This is a homozygous state showing almost exclusively HbS, although a small amount of HbF may also be present.

### Sickle-C Disease

This is a heterozygous state demonstrating HbS and HbC.

### Sickle Cell-Thalassemia Disease

This condition shows HbA, HbF, HbS, and HbA<sub>2</sub>.

In Sickle Cell  $\beta^0$ -Thalassemia HbA is absent.

In Sickle Cell  $\beta^+$ -Thalassemia HbA is present in reduced quantities.

### Thalassemia-C Disease

This condition shows HbA, HbF and HbC.

### C Disease

This is a homozygous state showing almost exclusively HbC.

### Thalassemia Major

This condition shows HbF, HbA and HbA<sub>2</sub>.

## SPECIFIC PERFORMANCE CHARACTERISTIC FOR SPIFE PRECISION

One control and one abnormal patient sample were tested for precision studies using two lots of gel.

**Within Run:** Each sample was run five times on one gel. N = 5

	HbA	HbF	HbS	HbC
<b>AFSC</b>				
Mean	25.6	26.5	27.6	20.2
SD	0.9	1.0	0.5	0.5
CV	3.4%	3.6%	1.9%	2.4%
	HbA	HbS	HbA <sub>2</sub>	
<b>Abnormal Patient</b>				
Mean	58.5	38.7	2.8	
SD	0.5	0.5	0.2	
CV	0.8%	1.2%	6.4%	

**Between Run:** Each of the two samples were run in replicate on four gels from two lots. N = 40

	HbA	HbF	HbS	HbC
<b>AFSC</b>				
Mean	25.3	26.4	27.8	20.5
SD	1.1	0.9	1.1	0.9
CV	4.3%	3.3%	3.9%	4.2%
	HbA	HbS	HbA <sub>2</sub>	
<b>Abnormal Patient</b>				
Mean	58.9	38.5	2.6	
SD	0.8	0.7	0.3	
CV	1.4%	1.9%	11.3%	

## CORRELATION

A correlation study of the QuickGel Alkaline Hemoglobin and the SPIFE Alkaline Hemoglobin was done using 50 normal and 50 abnormal specimens and three controls. The results were as follows:

N = 103 samples

Y = 1.017X - 0.789

R = 0.9978

X = SPIFE Alkaline Hb

Y = QuickGel Alkaline Hb

## SPECIFIC PERFORMANCE CHARACTERISTICS FOR QUICKGEL CHAMBER

### PRECISION

One control and one abnormal patient sample were tested for precision studies using two lots of gel.

**Within Run:** Each sample was run in replicate on one gel. N = 4

	HbA	HbF	HbS	HbC
<b>AFSC</b>				
Mean	29.1	25.4	26.8	18.7
SD	1.8	0.8	1.0	0.9
CV	6.2%	3.3%	3.8%	5.0%
	HbA	HbS	HbA <sub>2</sub>	
<b>Abnormal Patient</b>				
Mean	59.3	38.6	2.1	
SD	2.0	1.8	0.2	
CV	3.4%	4.7%	10.6%	

**Between Run:** Each of the two samples were run in replicate on three gels from one lot. N = 12

	HbA	HbF	HbS	HbC
<b>AFSC</b>				
Mean	29.1	26.3	27.0	17.6
SD	1.4	1.6	1.0	1.2
CV	4.7%	6.2%	3.5%	6.8%
	HbA	HbS	HbA <sub>2</sub>	
<b>Abnormal Patient</b>				
Mean	59.3	38.6	2.1	
SD	2.0	1.8	0.2	
CV	3.4%	4.7%	10.6%	

## CORRELATION

A correlation study of the QuickGel Alkaline Hemoglobin and the SPIFE Alkaline Hemoglobin was done using 25 normal and 25 abnormal specimens and one control. The results were as follows:

N = 51 samples

Y = 1.004X - 0.169

R = 0.9992

X = SPIFE Alkaline Hb

Y = QuickGel Alkaline Hb on QuickGel Chamber

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### QuickGel® ALKALINE HEMOGLOBIN SYSTEM

#### QuickGel Alkaline Hemoglobin Kit **Cat. No. 3429**

QuickGel Alkaline Hemoglobin Gels (10)  
Acid Blue Stain (1 vial)  
Hemolysate Reagent (25 mL)  
Citric Acid Destain (1 pkg)  
QuickGel Blotter C (10)  
QuickGel Blotter X (20)  
Applicator Blade Assembly-20 Sample (10)

#### QuickGel Alkaline Hemoglobin Kit **Cat. No. 3529**

QuickGel Alkaline Hemoglobin Gels (10)  
Acid Blue Stain (1 vial)  
QuickGel Blotter C (10)  
QuickGel Blotter X (20)  
Citric Acid Destain (1 pkg)  
Hemolysate Reagent (25 mL)  
QuickGel Applicator Blades (10)

#### QuickGel Alkaline Hemoglobin Kit

**Cat. No. 3529T**

QuickGel Alkaline Hemoglobin Gels (10)  
Acid Blue Stain (1 vial)  
QuickGel Blotter C (10)  
QuickGel Blotter A (10)  
QuickGel Blotter X (20)  
Citric Acid Destain (1 pkg)  
Hemolysate Reagent (25 mL)  
QuickGel Sample Templates (10)

#### Other Supplies and Equipment

The following items, needed for performance of the QuickGel Alkaline Hemoglobin Kits must be ordered individually.

ITEM	CAT. NO.
SPIFE 2000 Analyzer	1130
SPIFE 3000 Analyzer	1088
QuickScan 2000	1660
AFSC Hemo Control	5331
AA <sub>2</sub> Hemo Control	5328
REP Prep	3100
SPIFE QuickGel Electrodes	1111
SPIFE QuickGel Gel Holder	3358
SPIFE Gel Block Remover	1115
SPIFE QuickGel Chamber Alignment Guide	86541003
Applicator Blade Weights	3387
Disposable Sample Cups	3369
QuickGel Dispo Cup Tray	3353
Chamber Cover	8JP34012
QuickGel Chamber	1284
QuickGel Applicator	1265
QuickGel Applicator Base	1266
QuickGel Applicator Weights	1267
QuickGel Dispo Cup Tray	1268
QuickGel Dispo Sample Cups	1269
QuickGel Applicator Blades	1270
QuickGel Gel Block Remover	1262
QuickGel Applicator Kit	1274
(Includes Applicator, Applicator Base, Weights, and Cup Tray)	

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