

QuickGel® Touch Alkaline Hemoglobin Procedure

Cat. No. 3429

The QuickGel Touch Alkaline Hemoglobin method is intended for the qualitative and semi-quantitative determination of hemoglobins using agarose electrophoresis in alkaline buffer on the SPIFE Touch 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 HbA₂ 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 HbA₂ 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. Wintrobe¹ 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.²

Electrophoresis is generally considered the best method for separating and identifying hemoglobinopathies.³ The protocol for hemoglobin electrophoresis involves stepwise use of two systems.⁴⁻⁹ 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 Touch Alkaline Hemoglobin method 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 washed, 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% glacial 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. The stain must be replaced after processing ten gels to avoid contamination.

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: Discard if solution has precipitates or flocculent.

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 Touch analyzer must be used to apply samples, electrophorese, stain, destain and dry the gels. The gels may be scanned on a separate densitometer or the QuickScan Touch/2000 (Cat. No. 1690/1660). 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.
 4. After washing the samples, prepare the hemolysates by mixing 10 µL sample to 100 µL Hemolysate Reagent. Vortex or shake vigorously for 15 seconds.
- b) Control
 - AA₂ (Cat. No. 5328) no dilution is necessary
 - AFSC (Cat. No. 5331) 1:2 (1 part control + 1 part Hemolysate Reagent)

PROCEDURE

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)
Blade Applicator Kit (10)

Materials available but not contained in the kit:

Item	Cat. No.
SPIFE Touch	1068
QuickScan Touch	1690
QuickScan 2000	1660
AFSC Hemo Control	5331
AA ₂ 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 (Shallow Well)	3369
QuickGel Dispo Cup Tray	3353
Chamber Cover	8JP34012

Materials needed but not provided:

5% glacial acetic acid
0.85% NaCl

STEP BY STEP METHOD

I. Chamber Preparation

1. 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.
2. Remove the contact sheet and clean the chamber floor according to instructions in the Operator's Manual.
3. Place the round hole in the guide over the left chamber pin and the obround hole over the right pin.
4. 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

1. Prepare hemolysates of patient specimens and controls as instructed in the "Specimen Preparation" section.
2. Remove one Applicator Blade from the packaging.
3. Place the Applicator Blade into the vertical slots numbered 6 in the Applicator Assembly.

NOTE: The Applicator Blade will only fit into the slots in the Applicator Assembly one way; do not try to force the Applicator Blade into the slots.

4. Place an Applicator Weight on top of the Applicator Blade. When placing the weight on the blade, position the weight with the thick side to the right.
5. Slide the Disposable Sample Cup strip into the row numbered 1 to 10 of the Cup Tray.
6. Pipette 17 μ 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

1. 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.
2. Place a QuickGel Blotter C on the gel with the longer edge parallel with gel blocks. Gently blot the entire surface of the gel using slight fingertip pressure on the blotter and remove the blotter.
3. Dispense about 1 mL of REP Prep onto the left side of the marked area.

4. Place the gel over the REP Prep inside the rectangle on the chamber floor. Hold the gel so that the end numbered 1 to 10 is 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.
5. Clean the QuickGel electrodes with deionized water before and after each use. Wipe the electrodes with lint-free tissue.
6. 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.
7. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test and press **SETUP**.

IV. Electrophoresis/Staining

Using the instructions provided in the appropriate Operator's Manual, set up parameters as follows for the SPIFE Touch:

	Separator Unit
Load Sample	Prompt: None Time: 0:30 Temperature: 20°C Speed: 4
Apply Sample	Prompt: None Time: 0:30 Temperature: 20°C Speed: 4 Location: 1
Electrophoresis	Prompt: To Continue Time: 15:00 Temperature: 18°C Voltage: 550 V mA: 35 mA

End	
Stain	Stainer Unit Prompt: None Time: 4:00 Recirculation: Off Valve: 3 Fill, Drain
Destain 1	Prompt: None Time: 0:30 Recirculation: Rev Valve: 2 Fill, Drain
Dry 1	Prompt: None Time: 15:00 Temperature: 70°C
Destain 2	Prompt: None Time: 1:30 Recirculation: Rev Valve: 2 Fill, Drain
Destain 3	Prompt: None Time: 1:30 Recirculation: Rev Valve: 2 Fill, Drain
Dry 2	Prompt: None Time: 7:00 Temperature: 70°C
End	

1. Open the chamber lid. Place the Cup Tray with samples on the SPIFE Touch. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
2. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. The SPIFE Touch will apply the samples and beep when completed.
3. Open the chamber lid and 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.

4. Insert a Chamber Cover into the grooves of the chamber and close the chamber lid. Press **CONTINUE** to start electrophoresis.
5. At the end of the electrophoresis, the instrument will beep. Open the chamber lid and remove the Chamber Cover, electrodes and blotters. Dispose of the blade and cups as biohazardous waste.
6. 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

1. 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.
2. Place the Gel Holder with the attached gel facing backwards into the stainer chamber.
3. Use the arrows under **STAINER UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. The instrument will stain, destain and dry the gel.
4. 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.

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.

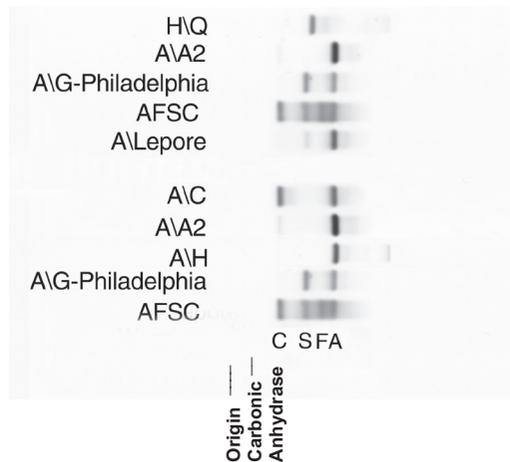
2. Quantitative evaluation: Determine the relative percent of each hemoglobin band by scanning the dried gels agarose side up on the QuickScan Touch/2000 using Acid Blue setting. A slit size of 4 is recommended. Verify that the default setting for "Smoothing" is "No". "Autoslope" may be used with this test.

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: AA₂ 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. Low Levels of HbF (1%-10%) may be accurately quantitated using any commercially available HbF method.

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 HbA₂ 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% HbA₂ and less than 2% HbF.³

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

HbA - 96.5% - 98.1%

HbA₂ - 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.² Several variants including HbH, E-Fort Worth and Lepore cause a thalassaemic blood picture.²

The two variant hemoglobins of greatest importance in the U.S., in terms of frequency and pathology, are HbS and HbC.² 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 α or β) while synthesis of the other chain proceeds normally.^{10,11} 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 α -thalassemias, the α chains are diminished or absent, and in the β -thalassemia, the β 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.¹¹

The most common hemoglobin abnormalities:

Sickle Cell Trait

This is a heterozygous state showing HbA and HbS and a normal amount of HbA₂ 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 HbA₂.

In Sickle Cell β^0 -Thalassemia HbA is absent.

In Sickle Cell β^+ -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 HbA₂.

PERFORMANCE CHARACTERISTICS

PRECISION

Within Run: A control and an abnormal patient were run alternately on a single gel with the following results:

	HbA	HbF	HbS	HbC
AFSC (n=4)				
Mean	33.8	21.9	24.4	20.0
SD	0.9	0.8	0.9	0.6
CV	2.6%	3.7%	3.6%	3.2%

	HbA	HbC
Abnormal Patient (n=3)		
Mean	58.7	41.3
SD	1.1	1.1
CV	1.9%	2.8%

Between Run: A control and an abnormal patient were run alternately on nine gels with the following results:

	HbA	HbF	HbS	HbC
AFSC (n=33)				
Mean	32.6	23.4	25.3	18.6
SD	1.4	1.4	1.2	1.3
CV	4.2%	6.1%	4.8%	7.1%

	HbA	HbC
Abnormal Patient (n=27)		
Mean	57.8	42.2
SD	1.2	6.1
CV	2.0%	2.8%

CORRELATION

Normal and abnormal specimens were analyzed using the QuickGel Alkaline Hemoglobin method on SPIFE 3000 and QuickGel Alkaline Hemoglobin method on the SPIFE Touch:

$$n = 8$$

$$Y = 0.9888X - 0.2851$$

$$R = 0.9997$$

$$X = \text{QuickGel Alkaline Hemoglobin on SPIFE 3000}$$

$$Y = \text{QuickGel Alkaline Hemoglobin on SPIFE Touch}$$

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