When dissolved as directed, the stain contains 0.25% EDTA and thimerosal as a preservative.

If storage is necessary, whole blood and packed cells may be stored up to 1 week at 2 to 8°C. Frozen packed cells may be stored for up to 4 months at -20°C.

The diluted stain should be a 1:20 dilution for analysis by agar electrophoresis, with the addition of a small amount of HbF. In addition, the reagent is used at a 1:10 dilution when separating and identifying hemoglobinopathies. The protocol for hemoglobin electrophoresis should employ use of two systems. "The solution is prepared as directed. Other supplies and equipment are needed for performance of the QuickGel Acid Hemoglobin Procedure. The reagent contains potassium citrate on cellulose acetate. Results on the gel should be interpreted with the appropriate Operator's Manual for detailed instructions.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INJECT - INTRAVENOUSLY.

Storage: The reagent must be stored at room temperature (15 to 30°C) and are stable until the expiration date indicated on the package. The dyed dilute stain is stable for up to 10 days at room temperature (15 to 30°C) and 10°C to 20°C for up to 2 weeks.

The QuickGel Acid Hemoglobin Electrophoresis Procedure is indicated for the identification of abnormal hemoglobins using a safe buffer in the SPIFE 2000 or the QuickGel Chamber. A negative reaction of the gel indicates that the sample is normal, whereas a positive reaction indicates the presence of an abnormal hemoglobin.

SUMMARY
Hemoglobin is a group of proteins whose chief function is to transport oxygen from the lungs to the tissues and carbon dioxide from the tissues back to the lungs. A specific region of abnormal hemoglobin synthesis (e.g., in HbC, HbS, and HbE) may cause serious clinical effects, especially in the homozygous state or in combination with another abnormal hemoglobin. The QuickGel Acid Hemoglobin procedure is a simple method for separating and identifying hemoglobinopathies. The protocol for hemoglobin electrophoresis should employ use of two systems. The reagent contains potassium citrate on cellulose acetate. Results on the gel should be interpreted with the appropriate Operator's Manual for detailed instructions.

REAGENTS
1. Acid Hemoglobin Stain: The reagent contains potassium citrate on cellulose acetate. Results on the gel should be interpreted with the appropriate Operator's Manual for detailed instructions.

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**STEP BY STEP METHOD**

### I. Chamber Preparation

1. Place the Applicator Blade into the vertical slot numbered 8 and force the Applicator Blade into the slots. Do not try to force the Applicator Blade into the slots in the Applicator Assembly one way; do not try to force the Applicator Blade into the slots.

2. Place an Applicator Blade Weight on top of each blade holder. This should fit into the slots in the Applicator Assembly one way; it will not fit if turned back to front.

3. Place the Applicator Blade into the vertical slot numbered 8 in the Chamber Assembly, making sure that the protective piece back and forth until it breaks free.

4. Place the Round Hole in the guide over the left chamber pin (numbered 1) of the appropriate pin if having more than 10 samples. Place the Sample Chamber Lid over the right chamber pin.

5. Place the Sample Chamber Lid over the right chamber pin.

**PROCEDURE FOR SPIFE 2000/3000**

**Materials needed but not provided:**

<table>
<thead>
<tr>
<th>ITEM</th>
<th>CAT. NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric Acid Destain (1 pkg)</td>
<td>3419</td>
</tr>
<tr>
<td>Acid Blue Stain (1 vial)</td>
<td>1151</td>
</tr>
</tbody>
</table>

**PROCEDURE FOR QuickGel CHAMBER**

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

<table>
<thead>
<tr>
<th>ITEM</th>
<th>CAT. NO.</th>
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<tbody>
<tr>
<td>Hemolysate Reagent (25 mL)</td>
<td>1284</td>
</tr>
<tr>
<td>Acetic Acid Destain</td>
<td>3419</td>
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</tbody>
</table>

**PROCEDURE FOR QuickGel**

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

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**PROCEDURE FOR SPIFE 2000**

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**PROCEDURE FOR QuickGel**

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**PROCEDURE FOR SPIFE 3000**

**Materials needed but not provided:**

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**PROCEDURE FOR QuickGel**

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

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<td>Acid Blue Stain (1 vial)</td>
<td>1151</td>
</tr>
</tbody>
</table>
The following materials needed for the procedure are contained in the QuickGel Acid Hemoglobin Kit (Cat. No. 9325). Additional materials available but not contained in the kit:

- 100 mM Acetate Buffer pH 4.6: 10 mL (Cat. No. 3519) or equivalent.
- Plastic Transparent Gloves: 1 pair (Cat. No. 1805).
- Gel Block Remover: 1 box (Cat. No. 1839).
- Disposable Sponge Cube: 10 blocks (Cat. No. 3707).
- Applicator Blade Assembly: 10 blades (Cat. No. 1270).
- QuickGel Chamber Alignment Guide: 1 each (Cat. No. 86541003).
- QuickGel Applicator Blades: 12 blades (Cat. No. 1267).
- QuickGel Applicator Weights: 12 weights (Cat. No. 1256).
- QuickGel Disposable Sample Cups: 10 cups (Cat. No. 0042).
- Disposable Sample Cups: 10 cups (Cat. No. 0042). If using 10 samples, remove 1 cup for each sample. If testing more than 10 samples, remove 2 cups for each sample. If testing more than 10 samples, remove 2 cups for each sample.

Additional requisites described in the “Specimen Preparation” section. If using only one quick gel, it should be placed into the slot corresponding to cup placement.

After electrophoresis is complete, open the chamber lid and place the Cup Tray with samples into the QuickGel Acid Hemoglobin Kit (Cat. No. 9325). If using two Applicator Blades, place the tray to the holder.

After 30 seconds, lift the Applicator Knob and immediately place the gel into the stain for 4 minutes. Remove the gel from the stain, and remove any excess staining by blotting the gel gently using a blotter.

Again use a gentle rocking and swirling technique. If using only one quick gel, it should be placed into the slot corresponding to cup placement.

3. Place the gel into the stain for 4 minutes. Remove the gel from the stain, and remove any excess staining by blotting the gel gently using a blotter.

8. Close the lid, press the power switch to turn on the QuickGel Chamber.

7. The procedure is complete. The stain will develop.

4. Place an Applicator Blade Weight on top of each blade to force the Applicator Blade into the slots. The QuickGel Chamber must be plugged into a power supply. Set a timer for 23:00 minutes and the power at 120 Volts, 40 mA. Enter the sample cups and immediately start a timer for 25:00 minutes or until dry.

1. Carefully cut open one end of the gel pouch. Remove the gel from the plastic backing to remove any excess REP Prep. Make sure that the gel is completely dry. Set a timer for 5:00 minutes.

5. After 30 seconds, lift the Applicator Knob and immediately place the gel into the stain for 4 minutes. Remove the gel from the stain, and remove any excess staining by blotting the gel gently using a blotter.

3. Open the chamber lid and place the Cup Tray with samples into the QuickGel Acid Hemoglobin Kit (Cat. No. 9325). If using two Applicator Blades, place the tray to the holder.
The following materials needed for the procedure are contained in the QuickGel Acid Hemoglobin Kit (Cat. No. 1284) for electrophoresis. These items are packaged in the QuickGel Acid Hemoglobin Gel. The hemoglobin gels should be inspected visually for the presence of abnormal hemoglobin bands. Glycated hemoglobin imitates with the Helena Laboratories’ Sickle-Thal Acid Hemoglobin Test kit. The hemoglobin gels should be run on the QuickGel Acid Hemoglobin Gel.

NOTE: A 3 "dry" time of 4 minutes and a 2 "time" of 4 minutes is a good starting point. However, in environmental conditions, the following ranges are acceptable.

<table>
<thead>
<tr>
<th>Electrophoresis/Staining</th>
<th>Sample</th>
<th>pH 5.8</th>
<th>pH 8.6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QuickGel Acid Hemoglobin</strong></td>
<td><strong>Sample</strong></td>
<td><strong>Sample</strong></td>
<td></td>
</tr>
<tr>
<td>Acidic</td>
<td>6.5</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>Basic</td>
<td>6.5</td>
<td>8.5</td>
<td></td>
</tr>
</tbody>
</table>

**Procedure**

1. Globin chain analysis (both acid and alkaline) and structural testing. Further testing is required if a particular hemoglobin concentration varies significantly from the normal range.

**Limitations**

Since abnormal hemoglobins have similar electrophoretic mobilities, further testing is generally required before a diagnosis is made. Further testing may include evaluation with other methods such as high-performance liquid chromatography (HPLC) and electrophoresis (capillary or slab gel).

**Evaluation of the Hemoglobin Bands**

The hemoglobin bands are visually inspected for the presence of abnormal hemoglobin bands.

**Quality Control**

The Helena Acid Hemoglobin Control (Cat. No. 5331) should be run with each gel. The Helena Acid Hemoglobin Control (Cat. No. 5331) is recommended. HbA2 and HbF in the presence of HbS, or the Helena Beta-Thalassemia trait. The Helena Laboratories' Sickle-Thal Acid Hemoglobin Test kit can be used for this purpose. 2.3.1.**REPRESENTATIVE SAMPLES**

1. Load Sample 1 00:30 20°C SPD. = 4
2. Dispense 0.5 mL REP Prep along the cathode side of the gel backing. Paint a line around the gel in the presence of HbS, or the Helena Beta-Thalassemia trait.
The diluted stain should be a clear, yellow

The reagent is ready for use as a screening method for the presence of abnormal hemoglobins using a dense buffer in the SPF-2000. (The SPF-2000 is also used when specific staining is required.)

The major hemoglobin in the erythrocytes of the normal adult is HbA, but there are small amounts of HbA

2 chains. Two other chains are formed in the embryo.

In Sickle Cell Anemia

In Sickle-C Disease

This condition shows HbA, HbF, HbS, and HbA

This is a homozygous state showing almost exclusively HbS, and HbA

Hemolytic anemias or erythrocytosis or if the heterozygote is affected. Another quantitative disorder of hemoglobin synthesis, hereditary persistent fetal hemoglobin (HPFH), is diminished or absent, and in the thalassemia syndromes, life long cyanosis, the Thalassaemia

The two mutant hemoglobins most commonly seen in the United States are HbS and HbC. Hb Lepore, HbE, HbG-Philadelphia,

The two most common cases of thalassemia are thalassemia major and thalassemia minor.

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The Tzanck smear is made by scratching a vesicle or an area of confluent erythema with a scalpel and squashing the cells on a glass slide. A dry film is prepared and stained with Wright's stain for the presence of abnormal leukocytes.

The thalassemias are a group of hemoglobin disorders characterized by hypochromia and microcytosis due to the deficiency or absence of one of the four polypeptide chains of normal hemoglobin.

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ACID HEMOGLOBIN SYSTEM

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This is a homozygous state showing almost exclusively HbS, and HbA

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The reagent is ready for use as pack aged.

**INTERPRETATION OF RESULTS**

Most hemoglobin variants cause no discernible clinical symptoms but are associated primarily to research studies. Variants are usually important when their presence leads to abnormal or sickle-like RBC morphology or HbF, HbA and HbF, which is the major hemoglobin present is less than 2% HbF.

This condition shows HbF, HbA and HbA.

**THALASSEMIA MAJOR**

In Sickle Cell Sickle-C Disease

This is a heterozygous state showing HbA and HbS and

**THE MOST COMMON HEMOGLOBIN ABNORMALITIES:**

no anemia.

for HPFH have normal development, are asymptomatic and have

gamma chain synthesis at about four months after birth which

unbalanced synthesis results in unstable globin chains. These

synthesis of the other chain proceeds normally.

diminished synthesis of one globin chain (the

characterized by hypochromia and microcytosis due to the

disease are characterized by hemolytic anemia that is milder than

mild hemolytic anemia which is attributed to the precipitation or

of the body. The individual with homozygous HbCC suffers

cell anemia (HbSS) is a cruel and lethal disease. It first manifests

The two variant hemoglobins of greatest importance in the U.S.,

E-Fort Worth and Lepore cause a thalassemic blood picture.

6. Schneider, R.G., et al., Abnormal Hemoglobins in a Quarter

5. Schneider, R.G., Methods for Detection of Hemoglobin

4. Center for Disease Control, Laboratory Methods for Detecting

BIBLIOGRAPHY

of abnormal hemoglobins using age in a stable buffer in the SPFE-2000, the

3. Hemolysate Reagent

Ingredients:

1. QuickGel Acid Hemoglobin Gel

hemoglobins in the sample are separated by electro phoresis

are automatically applied to the QuickGel Acid Hb gel. The

procedure requiring minute quantities of hemolysate to provide

electrical charge.

hemoglobins, the evaluation must be supplemented by citrate

mutants with minimal preparation time. However, because

separating and identifying hemoglobinopathies. The protocol

contains alpha and delta

The gels are ready for use as pack aged.

Storage and Stability:

The reagent is ready for use as pack aged.

The gels should be stored hori zontally

The gels are ready for use as pack aged.

C. Other Variants

This is a heterozygous state showing approximately HbA.

- Thalassemia Minor

This is a heterozygous state showing almost exclusively HbC.

- Thalassemia Major

This condition shows HbF, HbA and HbC.

- Thalassemia Major

This condition shows HbF, HbA and HbC.

QUICKGEL ACID HEMOGLOBIN SYSTEM

Cat. No. 3519

QUICKGEL HEMOGLOBIN TEST (10)

Acid Blue Stain

Acid Denat. Buffer

QuickGel Applicator Blades (10)

QuickGel Applicator Weights

Hemolysate Reagent (25 mL)

QuickGel Blotter C (10)

QuickGel Sample captive

Other Supplies and Equipment

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

Cat No.

3500-3000 Analyser

ATG-5 Henry Condor

Gel Bath warmer

REF Prep

Appl Stock 1
gel Electric

QuickGel Adj 1

QuickGel Adj 2

QuickGel Adj 3

Other Variants

This is a heterozygous state showing HbA, HbC and HbS and

This condition shows HbA, HbH, HbA and HbF in a balanced state.

This condition shows HbA, HbF, HbS and HbA in a balanced state. Rapid release of HbA from the RBC results in a state of near anemia.

This condition shows HbA, HbH, HbE and HbA.

This condition shows HbA, HbH, HbF and HbC.

This is a heterozygous state showing HbA, HbF, HbC and HbA.

This condition shows HbA, HbF, HbH and HbC.

This is a heterozygous state showing HbA, HbF, HbH and HbC.

This is a heterozygous state showing HbA, HbF, HbH and HbC.

This condition shows HbA, HbF, HbH and HbC.

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