QuickGel® ACID HEMOGLOBIN SYSTEM

QuickGel Acid Hemoglobin Kit
Cat. No. 3419

QuickGel Acid Hemoglobin Gels (10)

Acid Blue Stain (1 vial)
Hemolysate Reagent (25 mL)
QuickGel Blotter C (10)
Citric Acid Destain (1 pkg)
Blade Applicator Kit (10)

Other Supplies and Equipment

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

Item
Cat. No.
SPIFE Touch
1068
AFSC Hemo Control
5331
Gel Block Remover
1115
RESP Prep
3100
Applicator Blade Weights
3387
Disposable Sample Cups (Shallow Well)
3369
QuickGel Deps Cup Tray
3354
SPIFE QuickGel Electrode
1111
SPIFE QuickGel Holder
3356
SPIFE QuickGel Chamber Alignment Guide
8854003
Chamber Cover
8LP4012

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Helena Laboratories warrants its products to meet our published specifications and to be free from defects in materials and workmanship. Helena’s liability under this contract or otherwise shall be limited to replacement or refund of any amount not to exceed the purchase price attributable to the goods to which such claim is made. These alternatives shall be buyer’s exclusive remedies.

In no case will Helena Laboratories be liable for consequential damages even if Helena has been advised as to the possibility of such damages.

Storage and Stability:

The gels should be stored horizontally at room temperature (15 to 30°C) and is stable until the expiration date indicated on the vial. The reagent contains deionized water into the Destain vat. Add

Storage and Stability: The gels must be stored in the protective packaging in which they are shipped.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. Storage and Stability: The gels must be stored in the protective packaging in which they are shipped.

DO NOT REFRIGERATE OR FREEZE THE GELS

PROCEDURE

2. Acid Blue Stain
Ingredients: When dissolved as directed, the stain contains 0.5% (w/v) acid blue stain.
Warnings: 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.

Hemolysate Reagent
Ingredients: For the qualitative determination of abnormal hemoglobins using agar in acidic buffer on the SPIFE Touch.

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 opposite 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. The non-alpha chains of fetal hemoglobin are called gamma. A minor (3%) hemoglobin fraction called HbF contains alpha and delta chains. Two other chains are formed in the embryo.

Thalassemia-C Disease
In Sickle Cell

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Storage and Stability:

The gels should be stored horizontally at room temperature (15 to 30°C) and is stable until the expiration date indicated on the vial. The reagent contains deionized water into the Destain vat. Add

Storage and Stability: The gels must be stored in the protective packaging in which they are shipped.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. Storage and Stability: 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.

PRINCIPLE
Very small samples of hemolysates prepared from packed cells are automatically applied to the QuickGel Acid-Hb gel. The hemoglobin samples are separated by electrophoresis using a citrate buffer and are stained with Acid Blue Stain.

AGENTS
1. QuickGel Acid Hemoglobin Gels
Ingredients: Each gel contains agar in citrate buffer with 0.25% EDTA and thimerosal as a preservative.

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.

PROPRIETOR
Very small samples of hemolysates prepared from packed cells are automatically applied to the QuickGel Acid-Hb gel. The hemoglobin samples are separated by electrophoresis using a citrate buffer and are stained with Acid Blue Stain.

AGENTS
1. QuickGel Acid Hemoglobin Gels
Ingredients: Each gel contains agar in citrate buffer with 0.25% EDTA and thimerosal as a preservative.

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.
Warnings: 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 contains deionized water with 0.005 M EDTA, 0.175% saponin and 0.07% potassium cyanide.
WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT FITPITE 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% (v/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 must be used to electrophorese, stain, destain and dry the gels. 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. Frozen samples may produce an artifact band between HbA and HbA1c.

Preparation for Use: 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 to 5 volumes of normal saline solution (0.85% NaCl), centrifuging and aspirating supernatant as before.
4. After washing the samples, prepare the samples by mixing 10 µL sample to 100 µL Hémolyse Reagent, vortex or shake vigorously for 15 seconds.
5. Control
AFSC (Cat. No. 5331) 1:2 (1 part control + 1 part Hemolysate Reagent)

PROCEED WITH THE SPECIMEN

Materials provided: The following materials needed for the procedure are contained in the QuickGel Acid-Hb Kit (Cat. No. 3419). Individual items are not available:

QuickGel Acid Hemoglobin Gels (10) Acid Blue Stain (1 vial)
Materials available but not contained in the kit:

- SPIFE QuickGel Holder 3358
- SPIFE QuickGel Electrode 1111
- SPIFE QuickGel Chamber Alignment Guide 86541003
- Chamber Cover 8JP40412

Materials needed but not provided:
- 5% acetic acid
- 0.85% NaCl

IV. Electrophoresis/Staining

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

- **Load Sample**
  - Prompt: None
  - Time: 0:00
  - Temperature: 20°C
  - Speed: 4

- **Apply Sample**
  - Prompt: None
  - Time: 0:30
  - Temperature: 20°C
  - Speed: 4
  - Location: 1

- **Electrophoresis**
  - Prompt: To Continue
  - Time: 0:00
  - Temperature: 20°C
  - Voltage: 120 V
  - mA: 40 mA
  - Dry Prompt: Remove Gel Blocks
  - Time: 6:00
  - Temperature: 62°C

**Stainer Unit**

- **Stain**
  - Prompt: None
  - Time: 1:00
  - Recirculation: Off
  - Value: 3
  - Fill, Drain

- **Destain 1**
  - Prompt: None
  - Time: 1:00
  - Recirculation: Rev
  - Value: 2
  - Fill, Drain

- **Dry 1**
  - Prompt: None
  - Time: 6:00
  - Temperature: 63°C

- **Destain 2**
  - Prompt: None
  - Time: 2:00
  - Recirculation: Rev
  - Value: 2
  - Fill, Drain

- **Destain 3**
  - Prompt: None
  - Time: 2:00
  - Recirculation: Rev
  - Value: 2
  - Fill, Drain

- **Dry 2**
  - Prompt: None
  - Time: 6:00
  - Temperature: 63°C

**End**

1. Open the chamber lid and 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. Dispose of blades and cups as biohazardous waste.

3. Open the chamber lid and insert a Chamber Cover in the grooves of the chamber. Close the chamber lid.

4. Press **PRESSURE** to start electrophoresis.

5. After electrophoresis is complete, open the chamber lid and remove the Chamber Cover. Use the Gel Block Remover to remove the gel blocks. Place one electrode across each end of the gel to prevent curling during drying. Rinse the Chamber Cover before reuse.

6. Close the chamber lid and press the **CONTINUE** button to dry the gel.

**Visualization**

- After the gel has dried, carefully remove the gel from the electrophoresis chamber.

- Remove the SPIFE QuickGel Holder from the chamber. While holding the gel, squeeze on one side, 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 fit over the pins to secure the gel to the holder.

- Place the SPIFE QuickGel Holder with the attached gel facing backwards into the stainer chamber.

- 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.

- When the process is completed, the instrument will beep. Carefully remove the SPIFE Quick Gel 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**

The hemoglobin gels should be visually inspected for the presence of abnormal hemoglobin bands. Glycosylated hemoglobin migrates with HbF. The Helsinki AFC 3 Hemoglobin Control provides a marker for band identification.

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

**Quality Control:** The Helsinki AFC 3 Hemoglobin Control (Cat. No. 5331) should be run on each QuickGel Acid Hemoglobin Gel. The control verifies all phases of the procedure and acts as a marker to aid in the identification of the hemoglobins in the unknown samples.

**RESULTS**

Figure 1 illustrates the electrophoretic mobility of bands on the QuickGel Acid Hemoglobin Gel.
The dried gels are stable for an indefinite period

**STEP BY STEP METHOD**

**I. Chamber Preparation**
1. The SPIFE QuickGel Chamber Assembly 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 & clean the chamber floor according to instructions provided in the Operator’s Manual.
3. Place the round hole in the guide over the left chamber pin and the copper floor.
4. Using an indelible marker, outline the rectangular open area onto the floor. Gently lay the gel down on the REP Prep, starting from the left edge 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 GelElectrodes with deionized water before and after each use. Wipe with a lint-free tissue.
6. Place a GelElectrode onto the outside edge of each gel block inside the magnetic posts. Immerse the gel electrodes and the gel blocks 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.

**II. Electrophoresis/Staining**

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

<table>
<thead>
<tr>
<th>Load Sample</th>
<th>Prompt: None</th>
<th>Time: 1 minute</th>
<th>Temperature: 20°C</th>
<th>Speed: 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apply Sample</td>
<td>Prompt: None</td>
<td>Time: 0.30</td>
<td>Temperature: 20°C</td>
<td>Speed: 4</td>
</tr>
</tbody>
</table>

**Stain**

<table>
<thead>
<tr>
<th>Stain</th>
<th>Prompt: None</th>
<th>Time: 1 minute</th>
<th>Recirculation: Off</th>
<th>Value: 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Destain 1</td>
<td>Prompt: None</td>
<td>Time: 1.00</td>
<td>Recirculation: Rev</td>
<td>Value: 2</td>
</tr>
<tr>
<td>Destain 2</td>
<td>Prompt: None</td>
<td>Time: 2.00</td>
<td>Recirculation: Rev</td>
<td>Value: 2</td>
</tr>
<tr>
<td>Destain 3</td>
<td>Prompt: None</td>
<td>Time: 2.00</td>
<td>Recirculation: Rev</td>
<td>Value: 2</td>
</tr>
</tbody>
</table>

| Dry           | Prompt: Remove Gel Blocks | Time: 6:00 | Temperature: 62°C |

**III. Gel Preparation**

1. Carefully open one end of the pouch and remove one gel from the protective packaging. Reseal the pouch with slight pressure on the blotter, and remove the blotter.
2. Place a QuickGelBlotter 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 approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
4. Place the gel over the REP Prep inside the rectangle on the chamber floor. 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 GelElectrodes with deionized water before and after each use. Wipe with a lint-free tissue.
6. Place a GelElectrode onto the outside edge of each gel block inside the magnetic posts. Immerse the gel electrodes and the gel blocks 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. Visualization**

1. After the gel has been dried, carefully remove the gel from the electrophoresis chamber.
2. Remove the SPIFE QuickGel Holder from the stainer chamber. While holding the gel sectionwise 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 fit over the small pins to secure the gel to the holder.
3. Place the SPIFE QuickGel Holder with the attached gel facing backwards into the stainer chamber.
4. Use the arrows under STAIN UNIT to select the appropriate test. Press START and choose an option to proceed. The instrument will stain, destain, and dry the gel.
5. When the process is completed, 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**

The hemoglobin gels should be inspected visually for the presence of abnormal hemoglobin bands. Glycated hemoglobin migrates with HbF. The Helena AFSC Hemo Control provides a marker for band identification.

**Stain Unit**

<table>
<thead>
<tr>
<th>Stain</th>
<th>Prompt: None</th>
<th>Time: 1 minute</th>
<th>Recirculation: Off</th>
<th>Value: 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Destain 1</td>
<td>Prompt: None</td>
<td>Time: 1.00</td>
<td>Recirculation: Rev</td>
<td>Value: 2</td>
</tr>
<tr>
<td>Destain 2</td>
<td>Prompt: None</td>
<td>Time: 2.00</td>
<td>Recirculation: Rev</td>
<td>Value: 2</td>
</tr>
<tr>
<td>Destain 3</td>
<td>Prompt: None</td>
<td>Time: 2.00</td>
<td>Recirculation: Rev</td>
<td>Value: 2</td>
</tr>
</tbody>
</table>

| Dry           | Prompt: Remove Gel Blocks | Time: 6:00 | Temperature: 62°C |

The hemoglobins should be positively identified using the appropriate test. The Helena AFSC® Hemo Control (Cat. No. 5331) should be run on each QuickGel Acid Hemoglobin Gel. The control verifies all phases of the procedure and acts as a marker to aid in the identification of the hemoglobins in the unknown samples.

**RESULTS**

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

**LIMITATIONS**

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

Further testing required:
1. Globin chain analysis (both acid and alkaline) and structural studies may be necessary in order to positively identify some of the more rare hemoglobins.
2. Anion exchange column chromatography is the most accurate method for quantitating HbA2, Helena Laboratories’ Sickle-Thal Quad Column Method (Cat. No. 5334) for quantification of HbA2, and the Helena Beta-Thal HbA, Quad Column Procedure (Cat. No. 5341) are recommended. HbA2 quantitation is one of the most important diagnostic tests in the diagnosis of β-thalassemia trait.
3. When a particular hemoglobin concentration varies significantly from the control, the migration will be affected.

**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 adulthood, the major hemoglobin present is HbA with up to 3.5% HbF and less than 2% HbS.

**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 HbS, HbS-D and HbS-α-Los Angeles lead to serious sickling disorders.

Several variants including HbH, E-Fort Worth and Lepore cause a thalassemic blood picture. The two major variants of greatest importance in the U.S. in terms of frequency and pathology are HbS and HbC. Sickle cell anemia (HbSS) is a cruel and lethal disease. It first manifests itself at about 5 to 6 months of age. The clinical courses present an amazing spectrum of pain and temperature crises, aplastic crises with anemia, listless period, lethargy and infant 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 and alpha globin chains. Cases of HbCC are characterized by hemolytic anemia that is rarer 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 β or α) while synthesis of the other chain proceeds normally. 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-thalassemia 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 (HFPH), represents a genetic failure of the mechanisms that turns off gene expression. Cases of HFPH disease are characterized by hemolytic anemia that is rarer than sickle-cell anemia.

The most common hemoglobin abnormalities are Sickle Cell Trait, Sickle Cell-Thalassemia Disease, HbS-C Disease and HbS-β0 Thalassemia (HbS-C Disease). This is a heterozygous state demonstrating HbA and HbC and a normal amount of HbF, usually in the presence of HbS, or the thalassemia trait. This is a heterozygous state showing HbA and HbS and a normal amount of HbF. This is a heterozygous state demonstrating HbA and HbC, and a small amount of HbF may also be present. This is a heterozygous state demonstrating HbS and HbC. This condition shows HbA, HbH, HbS, and HbF.
In Sickle Cell β-Thalassemia HbA is absent. In Sickle Cell β-Thalassemia HbA is present in reduced quantities.

**Thalassemia C Disease**
This condition shows HbA2 and HbA C deficiency.

**Thalassemia Major**
This condition shows HbF, HbA2, and HbA C.

**BIBLIOGRAPHY**

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**QUICKGEL® ACID HEMOGLOBIN SYSTEM**
QuickGel Acid Hemoglobin Kit Cat. No. 3419
QuickGel Acid Hemoglobin Gels (10) 1. Acid Blue Stain (1 vial)
2. Hemolysate Reagent (25 mL)
3. QuickGel Blottet C (10)
4. Citric Acid Destain (1 pkg)
5. Blade Applicator Kit (10)

**Other Supplies and Equipment**
The following items, needed for performance of the QuickGel Acid Hemoglobin Kit, must be ordered individually.
- Item
  - Cat. No.
  - SPIFE Touch 1068
  - AFSC Hem Control 5331
  - Gel Block Remover 1115
  - RESP Prep 3100
  - Applicator Blade Weights 3387
  - Disposable Sample Cups (Shallow Well) 3369
  - QuickGel Deps Cup Tray 3354
  - SPIFE QuickGel Electrode 1111
  - SPIFE QuickGel Holder 3356
  - SPIFE QuickGel Chamber Alignment Guide 8654003
  - Chamber Cover 1LPS4012

**SPECIFICATIONS**
For technical and product specifications, contact Helena Laboratories.

**PROCEDURE**
**Preparation for Use:**
-**Materials provided:** The following materials needed for the procedure are contained in the QuickGel Acid Hemoglobin Kit (Cat. No. 3419). Individual items are not available.

**Reagents**
1. QuickGel Acid Hemoglobin Gels
   - Ingredients: Each gel contains agar in citrate buffer with 0.25% EDTA and thimerosal as a preservative. 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 stability of the gels is determined by the expiration date on the package. The expiration date is the last day of the current month indicated on the package.

2. Acid Blue Stain
   - Ingredients: When dissolved as directed, the stain contains 0.5% (w/v) acid blue.
   - 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 stability of the dry stain is determined by the expiration date on the package. The stability of the dry stain is determined by the expiration date on the package.

3. Hemolysate Reagent
   - Ingredients: The reagent contains deionized water with 0.005 M EDTA, 0.175% saponin and 0.07% potassium cyanide.
   - WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INJECT BY MOUTH. The reagent contains potassium cyanide.
   - Preparation for Use: The reagent is ready for use as packaged.

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 - IRITANT.
   - Preparation for Use: Pour 1 L of deionized water into the Destain vial. 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 is used to electrophorese, stain, destain and dry the gels. 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 at 1°C to 10°C for up to 7 days. Frozen samples may be stored for a maximum of 2 months at -15°C to -30°C.

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

1. Whole Blood sample
   - 1. Centrifuge anticoagulated blood for 10 minutes to separate cells from plasma.
   - 2. Remove plasma.
   - 3. Wash packed cells 3 times with resuspension in 5 to 10 volumes of normal saline solution (0.85% NaCl), centrifuging and aspirating supernatant as before.
   - 4. After washing the samples, prepare the samples by mixing 10 mL of sample with 100 µL Hemolysate Reagent, vortex or shake vigorously for 15 seconds.
   - 5. Control
     - 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 Acid Hemoglobin Kit (Cat. No. 3419). Individual items are not available.

**QuickGel Acid Hemoglobin Gels (10) Acid Blue Stain (1 vial)