The reagent contains deionized water with 0.005 M HC1. After dissolution, the destain contains 0.3% (w/v) citric acid.


BIBLIOGRAPHY
2. Farkas, V.F., Diagnostic Medicine, No.58, 1960.
The dried gels are stable for an indefinite time.

**Place the left edge of the gel over REP Prep aligning the round hole on the left pin. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right, fitting the obround hole over the right pin. Use lint-free tissue to wipe around the edges of the gel backing, especially next to electrode posts, to remove excess water.**

**Dispense approximately 2 mL of REP Prep onto left side of SPIFE disposable cup tray.**

**Place an Applicator Blade Weight on top of each Applicator Blade.**

**Place the Applicator Blade into the numbered vertical slot numbered 8 in the Applicator Assembly.**

**Place the Disposable Sample Cups into the stainer chamber.**

**Clean the electrodes with deionized water and wipe with lint-free tissue before and after use.**

**HbF migrates with HbA, and a small amount of HbA 2 may be present.**

**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 lifelong disease that first manifests itself at about 5-6 months of age.**

**Use the arrows under SEPARATOR UNIT to select the appropriate test.**

**Dispose of blades and cups as biohazardous waste.**

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

**Load Sample**
- Prompt: None
- Time: 0:50
- Temperature: 20°C
- Sped: 4
- Location: 1

**Apply Sample**
- Prompt: None
- Time: 0:50
- Temperature: 20°C
- Sped: 4
- Location: 1

**Electrophoresis**
- Prompt: None
- Time: 1:00
- Temperature: 17°C
- Voltage: 250 V
- mA: 85 mA
- Temperature: 20°C
- Speed: 4
- Location: 1

**Dry**
- Prompt: Remove Gel Blocks
- Time: 4:00
- Temperature: 62°C
- Sped: 4
- Location: 1

**Stain**
- Prompt: None
- Time: 4:00
- Temperature: 37°C
- pH: 7.0
- Voltage: 250 V
- Sped: 4
- Location: 1

**Destain 1**
- Prompt: None
- Time: 2:00
- Temperature: 54°C
- Voltage: 250 V
- Sped: 4
- Location: 1

**Destain 2**
- Prompt: None
- Time: 2:00
- Temperature: 54°C
- Voltage: 250 V
- Sped: 4
- Location: 1

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

**Quality Control:**
- The Helena AFSC Hemo Control (Cat. No. 5331) should be run on each SPIFE 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 SPIFE Acid Hemoglobin Gel. Touch will apply the samples, electrophoresis and beep when complete.

**LIMITATIONS**
- Some abnormal hemoglobins have similar electrophoretic mobilities and must be differentiated by other methodologies. Further testing required:
  - Globin chain analysis (both acid and alkaline) and structural studies may be necessary in order to positively identify some of the more rare hemoglobin.
  - Anion exchange column chromatography is the most accurate method for quantitating HbA2, Helena Laboratories’ Sickle-Thal Quik Column Method (Cat. No. 5334) for quantitation of HbA and HbS in the presence of HbC and the Helena Beta-Thal-HbA2, Quik Column Procedure (Cat. No. 5341) are recommended. HbA, quantitation is one of the most important procedures in the diagnosis of thalassemia trait.
  - When a particular hemoglobin concentration varies significantly from the control, the migration will be affected.

**REFERENCE VALUES**
- 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 anemia, erythrocytosis or if the heterozygote is of sufficient prevalence to warrant genetic counseling. The combinations of HbS-S, HbS-D, HbD and HbO-Arab lead to serious sickling disorders.
- Several variants, including Hbh, E-Fort Worth and Lepore, cause a thalassemic picture.

**Other Variant Hemoglobins**

- The most common hemoglobin abnormalities: Sickle Cell Trait

**Sickle Cell Trait**
- This is a heterogeneous state showing HbA and HbS and a normal amount of HbA 2 on cellulose acetate. Results on citrate agar show hemoglobins in the HbA and HbS migratory positions (zones).

**Sickle Cell Anemia**
- This is a homogenous state showing almost exclusively HbS, although a small amount of HbA 2 may also be present.
STEP BY STEP METHOD

I. Sample Preparation
1. Prepare lysates of patient specimens and controls as instructed in the "Preparation" section.
2. Place the Applicator Blade into the numbered vertical slot numbered in the Applicator Assembly.

NOTE: 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.

3. Place an Applicator Blade Weight on top of each Applicator Blade.
4. Place the Disposable Sample Cups into the center channel of the SPIFE Disposable Cup Tray.

II. Gel Preparation
1. Remove the gel from the protective packaging and discard the overlay.
2. Place a REP Blocker C on the gel with the longer edge parallel to the gel blocks. Gently blot the entire surface of the gel using slight fingertip pressure on the blotter and remove the blotter.
3. Dispense approximately 2 mL of REP Prep onto left side of SPIFE Disposable Cup Tray. Cover until ready for use.
4. Place the left edge of the gel over REP Prep aligning the round hole on the left pin. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right, fitting the obround hole over the right pin. Use lint-free tissue to wipe around the edges of the gel backing, especially next to electrode posts, to remove excess REP Prep. Make sure that the gel lays flat in the chamber and that no bubbles remain under the gel.
5. Clean the electrodes with deionized water and wipe with lint-free tissue before and after use.
6. Place a carbon electrode on the outside ledge of each gel block outside the magnetic posts. Improper contact between the electrode and the gel block may cause skewed patterns. Close chamber lid.
7. Use the arrows under SEPARATOR UNIT to select the appropriate test. To check parameters, select test and press SETUP.

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

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IV. Electrophoresis
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, electrophoresis and beep when complete. Dispose of blades and cups as biohazard waste.
3. After electrophoresis is complete, use the Gel Block Remover to remove the gel blocks. Place one electrode across each end of the gel to prevent curling during drying.
4. Close the chamber lid and press the CONTINUE button to dry the gel.

V. Visualization
1. After the gel has been dried, carefully remove the gel from the electrophoresis chamber.
2. Remove the Gel Holder from the sample chamber. Attach the gel to the holder by placing the hole over the left pin and the obround hole over the right pin. Gently lay the gel down over the REP Prep. Make sure that the gel lays flat in the chamber and that no bubbles remain under the gel.
3. Clean the electrodes with deionized water and wipe with lint-free tissue before and after use.
4. Place a carbon electrode on the outside ledge of each gel block outside the magnetic posts. Improper contact between the electrode and the gel block may cause skewed patterns. Close chamber lid.
5. Use the arrows under SEPARATOR UNIT to select the appropriate test. To check parameters, select test and press SETUP.

VI. 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.

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

Quality Control: The Helena AFSC Hemo Control (Cat. No. 5331) should be run on each SPIFE Disposable Cup Tray. The control verifies all phases of the procedure and acts as a marker to aid in the identification of the hemoglobin in the unknown samples.

RESULTS

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

LIMITATIONS

Some abnormal hemoglobins have similar electrophoretic mobilities and must be differentiated by 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 Qual Column Method (Cat. No. 5334) for quantitation of HbA, in the presence of HbS or the Helena Beta-Thal HbA2, Qual Column Procedure (Cat. No. 5341) are recommended. HbA1c is one of the most important diagnostic tests in the diagnosis of thalassemia trait.
3. When a particular hemoglobin concentration varies significantly from the control, the migration will be affected.

REFERENCE VALUES

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 anemia, erythrocytosis or if the heterozygote is of sufficient prevalence to warrant genetic counseling. The combinations of HbS-H, HbS-D-Los Angeles and HbS-O-Arab lead to serious sickling disorders. Several variants, including HhH, E-Fort Worth and Lepore, cause a thalassemic blood picture.

The two variant hemoglobins of greatest importance in the U.S., in terms of frequency and pathology, are Hbs HbC and HbS. Sickle cell anemia (HbSS) is a lethal disease that 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 infant in virtually all organs of the body. The individual with congenital 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 or more globin chains (the α or β) while synthesis of the other chain proceeds normally. 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-thalassemias, 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 4 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.

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 HbA2 may also be present.
The SPIFE Touch Acid Hemoglobin Electrophoresis procedure is intended for the qualitative determination of hemoglobins using agar in acidic buffer on the SPIFE Touch system.

**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. The non-alpha chains of fetal hemoglobin are called gamma. A minor (3%) hemoglobin fraction called HbA includes alpha and delta chains. Two other chains are formed in the embryo. The major hemoglobin in the erythrocytes of a 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. Wintrobe divided 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 hemoglobin most commonly seen in the United States are HbS and HbC. Hb Lepore, HbE, HbG-Philadelphia, HbD-Los Angeles 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 may show other properties than electrical charge. This method is based on the complex interactions of the hemoglobin with 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 may show other properties than electrical charge. This method is based on the complex interactions of the hemoglobin with

**PRINCIPLE**

Very small quantities of lysates prepared from washed, packed cells are automatically applied to the SPIFE Acid Hb gel. The hemoglobins in the sample are separated by electrophoresis using a citrate buffer and are stained with Acid Blue Stain.

**REAGENTS**

1. SPIFE Acid Hb Gel

   **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.**

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