The SPIFE 4000 Acid Hemoglobin method is intended for the qualitative determination of hemoglobins using agar in acidic buffer on the SPIFE 4000 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 the search of four polypeptide chains. Each normal hemoglobin molecule contains one pair of alpha and one pair of non-alpha chains. The alpha and beta chains of the non-fetal hemoglobin are called gamma. A minor (3%) hemoglobin fraction called HbA 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, but there are small amounts of HbA, F, S, and C. 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 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. In homozygous state or in combination with another abnormal hemoglobin, Wintrobe 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, and HbG, in the United States are HbS and HbC. Hb Lepore, HbE, and HbG, in other parts of the world are HbS and HbC. Hb Lepore, HbE, and HbG, may be seen in the United States.

**Storage and Stability:** Store the Destain at 15 to 30°C and is stable until the expiration date indicated on the package. The diluted stain is stable one year 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. 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 vial. Add the entire package of Destain. Mix well until completely dissolved. Pour the entire contents of the Destain additive bottle into the prepared Destain and mix.

**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.

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**BIBLIOGRAPHY**


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Helena Laboratories warrants its products to meet our published specifications and to be free from defects in material and workmanship. Helena’s liability under this contract or otherwise shall be limited to replacement of refund of any amount not to exceed the purchase price attributable to the goods as 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. The foregoing warranties are in lieu of all warranties expressed or implied including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose.
IV. Electrophoresis

1. Click START on the screen and respond to the analyzer prompts. The analyzer will receive samples, electrophorese, stain, destain and dry the gel(s).
2. The cassette with the gel will be dropped into the cassette receptacle.
3. Remove the cassette(s) from the receptacle. If gel storage is required, remove and discard the two gel blocks.
4. Clean or wipe the non-gel side. If not, discard the used gels, applicator blades and sample trays as biohazardous waste.
5. Cassettes and carbon electrodes should be washed and dried after each use with deionized water. Refer to the Operator’s Manual for instructions.

Evaluation of the Hemoglobin Bands

The hemoglobin gel should be inspected visually for the presence of abnormal hemoglobin bands. Glycated hemoglobin migrates with HbF. The Helena AFS-C 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 AFS-C Hemo Control (Cat. No. 5331) should be run on each SPIFE 4000 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 mobilities of bands on the SPIFE 4000 Acid Hemoglobin Gel.

REFERENCE VALUES

At birth, the majority of hemoglobin in the erythrocytes of the normal newborn is fetal hemoglobin, HbF. Some of the major adult hemoglobin, HbA, and a small amount of HbA, are also present. At the end of the first year of life and through adulthood, HbA and HbA are the predominant adult hemoglobin.

INTERPRETATION OF RESULTS

Most hemoglobinopathies cause no discernible clinical symp- toms, so are of interest primarily to researchers. Variants are clinically important when their presence leads to sickling disorders, thalassemia, life long anemia, hemolytic anemia or erythrocytosis or if the heterozygote is of sufficient prevalence to warrant genetic counseling. The combinations of HbS, HbD-Los Angeles, and some others lead to serious sickling disorders. Several variants including HbH, 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 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 course presents many episodes of pain and temperature elevations with anemia, life threatening, lethargy, and fainting in virtually all organs of the body. The individual with homozygous HbSS suffers mild to moderately severe anemia which is attributed to the precipitation or crystallization of HbS within the erythrocytes. Cases of HbS disease are characterized by hemolytic anemia that is milder than sickle-cell anemia.

The thalassemias are a group of hemoglobin disorders charac- terized by hypochromia and microcytosis due to the diminished synthesis of one globin chain (the δ or μ) 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-thalassemias, the α-chains are diminished or absent, and in the β-thalassemias the β-chains are affected. Another quantitative disorder of hemoglobin synthesis, heredi- tary persistence of 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. The most common hemoglobin abnormalities are:

Sickle Cell Trait

This is a heterogeneous state showing HbA and HbS and a normal amount of HbA, 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 HbA may also be present. Sickle Cell Trait

This is a heterogeneous state demonstrating HbS and HbC.

Sickle Cell-Thalassemia Disease

This condition shows HbA, HbF, HbB and HbA. 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 HbA, HbF and HbC.

C Disease

This is a homozygous state showing almost exclusively HbC.

Thalassemia Major

This condition shows HbF, HbA and HbA.
The dried gels are stable for an indefinite period of time.

**SPECIMEN COLLECTION AND HANDLING**

**Sample and Gel Preparation**

- **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 (0.85% NaCl), centrifuging and aspirating supernatant.
  4. After washing the samples, prepare the samples by mixing 10 µL samples with 0.3% acetic acid.

- **B) Control**
  
  The AFSC Control (Cat. No. 5331) 1:2 (1 part control + 1 part Hemolysate B) Control must be prepared for each patient specimen.

**Stage**

- **Pre-Dry**
  - **Dry**

- **Dry**
  - **Cycles**
  - **A) Maximum of 3 gels can be run at a time.**

**Electrophoresis Parameters**

- **Sample Application**
  - **Applicator Load Time (mm:ss)**
  - **Applicator Load Speed**
  - **Application Rows**
  - **Application Cycles**
  - **Absorption Time (mm:ss)**
  - **Inter- Gel Start Delay (mm:ss)**

- **Electrophoresis Voltage**
- **Minimum Current (mA)**
- **Maximum Current (mA)**
- **Temperature (°C)**
- **Time (hh:mm:ss)**
- **Time (hh:mm:ss)**
- **Absorption Time (mm:ss)**
- **Time (hh:mm:ss)**

**REFERENCE VALUES**

At birth, the majority of hemoglobin in the erythrocytes of the newborn 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 throughout adulthood, the adult hemoglobin (HbA) present is HbA with up to 3.5% HbA2 and less than 2% HbF.

**INTERPRETATION OF RESULTS**

Most hemoglobins cause no discernible clinical symptoms, so are of interest primarily to research scientists. Variants are clinically important when their presence leads to sickling disorders, unstable hemoglobin, life-long anemia, hemolytic anemia or erythrocytosis or if the heterozygote is of sufficient prevalence to warrant genetic counseling. The combinations of HbS, HbS-D-Los Angeles and HbS-HbC lead to serious sickling disorders. Several variants including HbH, E-Fort Worth and Lepore cause athalassemic blood picture. The two variant hemoglobins 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 course presents an 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 hyponcromia and microcytosis due to the diminished synthesis of one globin chain (the ß or ß) while synthesis of the other chain remains at normal levels. 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 ß-thalassemias the ß-chains are diminished or absent, and in the ß-thalassemias the ß-chains are absent. Another quantitative disorder of hemoglobin synthesis, hereditary persistence of 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. The most common hemoglobin abnormalities:

**Sickle Cell Trait**

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

**Sickle Cell Disease**

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

**Sickle Cell Trait**

This is a heterozygous state demonstrating HbS and HbC.

**Sickle Cell-Thalassemia Disease**

This condition shows HbA, HbF, HbS and HbA2.

**Thalassemia Major**

This condition shows HbF, HbA, and HbA2.

**Thalassemia Major**

This is a homozygous state showing almost exclusively HbC.

**Thalassemia Major**

This condition shows HbF, HbA, and HbA2.

**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 hemoglobin.
- 2. When a particular hemoglobin concentration varies significantly from the control, the migration will be affected.

**STEP BY STEP METHOD**

1. **Prepare SPIFE 4000 Applicator Blades (one per gel)**
2. **Fill the designated bottles with deionized water and destain.**
3. **Add prepared acid blue stain to the proper sample bottle.**
4. **Carefully open one end of the pouch, remove the gel from the protective package and discard the overlay.**
5. **Use a SPIFE 4000 Blotter C, gently blot the entire gel. Discard the blotter.**
6. **Hold the gel so that the barcode is at the top. Place the gel into the cassette by holding the gel backing in one hand and gently bending the gel. Slide each end of the gel backing into the slots of the cassette to hold it in place.**
7. **Slide the cover in the slot so that the lower unit. Click the SPIFE 4000 icon on the screen and respond to the analyzer prompts.**
8. **Place the cassette with the gel into the humidor and cover. The hemoglobin gel should be inspected visually for the presence of abnormal hemoglobin bands. Glycated hemoglobin migrates with HbF. The Helena HAFSC Hemo Control provides a marker for band identification.**
9. **The stain must be replaced after processing ten gels to avoid contamination.**
10. **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 4000 Acid Hemoglobin Gel.
The SPIFE® 4000 Acid Hemoglobin Method is intended for the qualitative determination of hemoglobins using agar in acidic buffer on the SPIFE® 4000 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 ion protoporphyrin heme groups. A specific sequence of amino acids constitutes the search 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 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, but 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® 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. HbLepore, HbE, HbH, Philadelphia, HbD-Los Angeles and HbO-Arab may be seen less frequently.

Electrophoresis is generally considered the best method for separating and identifying hemoglobinopathies. The protocol for hemoglobin electrophoresis involves stepwise use of two systems. After initial electrophoresis is performed in alkaline buffers, cellulose acetate was the major support medium used because it 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 acid electrophoretic buffer and the agar support. The SPIFE 4000 Acid 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 automatically applied to the SPIFE 4000 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 4000 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 horizontal 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 precipitation of the agar, (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-VITO DIAGNOSTIC USE ONLY. DO NOT INGEST.

Preparation for Use: Dissolve the dry stain (entire contents of vial) in 1 L of 0.5% 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 one year 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 contains deionized water with 0.005 M EDTA, 0.175% saponin and 0.07% potassium cyanide.

WARNING: FOR IN-VITO DIAGNOSTIC USE ONLY. DO NOT PIPEETE 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 is foculent.

4. Citric Acid Destain

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

WARNING: FOR IN-VITO 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. Pour the entire contents of the Destain Additive bottle into the prepared Destain and mix.

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.

5. Destain Additive

Ingredients: The product is a wetting agent.

WARNING: FOR IN-VITO DIAGNOSTIC USE ONLY. DO NOT INGEST - IRRITANT.

Preparation for Use: Pour the entire contents of the Destain Additive bottle into 11 L of prepared Citric Acid Destain.

Storage and Stability: The additive should be stored at 15 to 30°C and is stable until the expiration date indicated on the package.