The gels are ready for use as pack
When dissolved as directed, the stain contains
The reagent contains deionized water with 0.005

BIBLIOGRAPHY


The SPIFE 4000 Acid Hemoglobin Electrophoresis Procedure 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 function is 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 these polypeptide chains. Each normal hemoglobin molecule contains one pair of alpha and one pair of non-alpha chains. The theta-alpha chains of 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 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 hemoglobins most commonly seen in the United States are HbS and HbC. Hb Lepra, 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 step-wise use of two systems.

Initial electrophoresis is performed in alkaline buffers. Cellulosic 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 Procedure is a simple procedure requiring minute quantities of hemolsate to provide a screening method for the presence of abnormal hemoglobins such as HbS, HbC, and HbE.

PRINCIPLE

Very small samples of hemolysates prepared from packed cells are automatically applied to the SPIFE 4000 Acid Hb gel. The hemoglobins in the sample are separated by electrophoresis using a citrate agar 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 pack-aged.

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. 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) thickening 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% 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.

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 pack-aged.

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. 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.

5. Destain Additive

Ingredients: The product is a wetting agent.

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

Preparation for Use: Pour the entire contents of the Destain Additive bottle into the 1 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.

Signs of Deterioration: The additive should be free of precipitate.
The following materials needed for the procedure are contained in the SPIFE 4000 Acid Hemoglobin Gel Kit (Cat. No. 9325). Individual items are not available.

**Materials provided:**
- SPIFE 4000 Acid Hemoglobin Gels (10)
- Acid Blue Stain (1 vial)
- Citric Acid Destain (1 pkg)
- SPIFE 4000 Blotter C (10)
- SPIFE 4000 Applicator Blades (10)
- Destain Additive (28 mL)
- Operator's Manual

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

**STEP BY STEP METHOD**

### I. SPIFE Preparation

1. Place SPIFE 4000 Applicator Blades (one per gel) beginning in the first position of the Application tray.
2. Fill the designated wells with deionized water and destain.
3. Add prepared acid blue stain to the appropriate stain bottle.
4. Centrifuge anticoagulated blood for 10 minutes to separate cells from plasma.
5. Remove plasma.
6. Wash packed cells 3 times by resuspending in 5 to 10 volumes of normal saline solution (0.85% NaCl) centrifuging and aspirating supernatant as before.
7. After washing the samples, prepare the samples by mixing 10 μL sample to 100 μL Hemolysate Reagent. Vortex for 15 seconds.
8. Centrifuge at 2000 rpm for 5 minutes.
10. Discard the supernatant.
11. Wash the gel(s) with 5% acetic acid for 20 minutes.
12. Destain with citric acid destain for 20 minutes.
13. Repeat steps 9 through 12 until clear.
14. Air dry gel(s).

**Materials needed but not provided:**
- SPIFE 4000 Acid Hemoglobin Gels (10)
- Acid Blue Stain (1 vial)
- Citric Acid Destain (1 pkg)
- SPIFE 4000 Blotter C (10)
- SPIFE 4000 Applicator Blades (10)
- Destain Additive (28 mL)

**PROCEDURE**

1. Prepare hemolysates of patient specimens and controls as instructed in the “Specimen Preparation” section.
2. Place the hemolysate solution (1 mg/mL) into each sample cup in the top row. Load samples in cups 1-7, skip the smaller cup in the center of the row and then load samples in cups 8-14.
3. Stain the appropriate number of disposable sample trays, with samples loaded, into the sample tray holder (one tray per gel), placing the first tray on the bottom. Place an additional empty sample tray on top of the stack to prevent evaporation of sample.
4. Carefully open each well of the pouch, remove the gel from the protective package, and discard the overlay.
5. Using a SPIFE 4000 Blotter C, gently blot the entire gel. Discard the blotter.
6. Hold the gel so that the bar code 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 over the top and bottom of the cassette holder in place. Align the cut out in the gel backing with the alignment pin in the cassette.
7. Select the gel block makes good contact with the electrodes to prevent skewed patterns.
8. Place the cassette with the gel into the humidifier and cover the topmost cassette with the Gel Staging Lid. Close the humid lid to minimize gel dehydration.
9. Follow the steps 4-8 for each gel needed.

**NOTE:** A maximum of 3 gels can be run at a time.

### II. Sample and Gel Preparation

#### A) Whole Blood sample

-Centrifuge anticoagulated blood for 10 minutes to separate cells from plasma.
-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 as before.
-After washing the samples, prepare the samples by mixing 10 μL sample to 100 μL Hemolysate Reagent. Vortex for 15 seconds.
-3 Centrifuge at 2000 rpm for 5 minutes.
-3 Precipitate and aspirate supernatant.
-3 Discard the supernatant.
-3 Wash the gel(s) with 5% acetic acid for 20 minutes.
-3 Destain with citric acid destain for 20 minutes.
-3 Repeat steps 9 through 12 until clear.
-3 Air dry gel(s).

#### B) Control

-Place SPIFE 4000 Applicator Blades (one per gel) begin
-3. Add prepared acid blue stain to the appropriate stain bottle.
-3. Wash packed cells 3 times by resuspending in 5 to 10 volumes of normal saline solution (0.85% NaCl) centrifuging and aspirating supernatant as before.
-3. After washing the samples, prepare the samples by mixing 10 μL sample to 100 μL Hemolysate Reagent. Vortex for 15 seconds.
-3. Centrifuge at 2000 rpm for 5 minutes.
-3. Precipitate and aspirate supernatant.
-3. Discard the supernatant.
-3. Wash the gel(s) with 5% acetic acid for 20 minutes.
-3. Destain with citric acid destain for 20 minutes.
-3. Repeat steps 9 through 12 until clear.
-3. Air dry gel(s).

#### III. Electrophoresis Parameters

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</tbody>
</table>

**LIMITATIONS**

Some abnormal hemoglobins have similar electrophoretic mobilities and must be differentiated by other methodologies. Further testing is recommended:

1. Glycated hemoglobin analysis (both acid and alkaline) and structural studies may be necessary in order to positively identify some of the more rare hemoglobin.
2. Anion exchange column chromatography is the most accurate method for quantitating HBa.

**REFERENCE VALUES**

At birth, the majority of hemoglobin in the erythrocytes of the normal full-term infant is fetal hemoglobin, HBF. Some of the major adult hemoglobins, HbA and HbA2, are not detectable at birth. The newborn is especially vulnerable to the adverse effects of anemia. Decreased oxygen delivery to the tissues of the body. The individual with homozygous HbA2 disease suffers from mild hemolytic anemia that is attributed to the precipitation or crystallization of HbA2 within the erythrocytes. Cases of HbA2 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. These 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 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 HbA2, and a small amount of HbS, are absent. At the end of the first year of life and through adulthood, the major hemoglobin present is HbA with up to 3.5% HbS, and less than 2% HBF.

**INTRODUCTION TO THE RESULTS**

Most hemoglobin variants cause no discernible clinical symptoms, so are of interest primarily to researchers. Variants are clinically important when their presence leads to sickling disorders, thalassemia syndromes, life long cyanosis, hemoglobin anemia or erythrocytosis or if the heterozygote is of sufficient prevalence to warrant genetic counseling. The combinations of Hb S-S, Hb S-D-Los Angeles and Hb S-S-Arab lead to serious sickling disorders. Several variants including Hb E, 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 manifests itself at about 5 to 6 months of age. The clinical course presents anorhagia, episodic painful crises and temperature elevations accompanied by anemia, listlessness, lethargy and infant in virtually all organs of the body. The individual with homozygous HbCC suffers from mild hemolytic anemia which is attributed to the precipitation or crystallization of HbC within the erythrocytes. Cases of HbC 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. These 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 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 HbA2, and a small amount of HbS, are absent. In Sickle Cell trait HbA2 is present.
I. Sample and Gel Preparation

1. Add prepared acid blue stain to the appropriate stain bottle.

2. Fill the designated bottles with deionized water and destain.

3. Stack the appropriate number of disposable sample trays, with samples loaded, into the sample tray holder (one tray per gel). Place the additional empty sample tray on top of the stack to prevent evaporation of samples.

4. Carefully open one end of the pouch, remove the gel from the protective packaging and discard the overlay.

5. Using a SPIFE 4000 Blotter C, gently blot the entire gel.

6. Hold the gel so that the barcode is at the top. Place the gel into the cassette by holding the gel back in one hand and gently bending the gel. Slide each end of the gel back into the slots of the cassette to hold it in place. Align the cut out in the gel backing with the alignment pin in the cassette.

7. Ensure the gel blocks make good contact with the electrodes to prevent skewed patterns.

8. Place the cassette with the gel into the humidor and cover.

III. Electrophoresis Parameters

Using the instructions provided in the Operator’s Manual, select:

- Test Type: Acid Hb
- Sample Application: Acid
- Stain: Destain
- Dry Sample Application: Applicator Load Time (mm:ss) 01:00
- Applicator Load Time 1
- Applicator Load Time 85
- Application Rows 1
- Row 1 Location (mm from gel edge) 55.0
- Apply Time (mm:ss) 01:00
- Apply Time Cycles 1
- Apply Time Inter-Gel Start Delay (mm:ss) 00:00
- Electrohoresis Voltage 160
- Minimum Current (mA) 10
- Maximum Current (mA) 100
- Temperature (°C) 18
- Time (hh:mm:ss) 00:00:00
- Pre-Dry Temperature (°C) 62
- Time (hh:mm:ss) 00:10:00
- Stain Time (hh:mm:ss) 00:00:00
- Stain Acid Blue
- Time (hh:mm:ss) 00:00:00
- Destain Agitate
- Time (hh:mm:ss) 00:00:00
- Dry Agitate
- Time (hh:mm:ss) 01:00

IV. Electrophoresis

1. Click START on the screen and respond to the analyzer prompts. The analyzer will apply samples, electrophoresis, stain, destain and dry the gels.

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. Clean or wipe the non-gel side. If not, discard the used gels, applicator blades and sample trays as biohazard waste.

4. Cassettes and carbon electrodes should be washed and dried after each use with deionized water. Refer to the Operator’s Manual for instructions.

V. 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. 3311) 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 mobility of bands on the SPIFE 4000 Acid Hemoglobin Gel.

LIMITATIONS

Some abnormal hemoglobins have similar electrophoretic mobilities and must be differentiated by other methodologies. Further testing may be necessary in order to positively identify some of the more rare hemoglobins.

1. Gluton 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’ Speci-Test QuikPlate Hemoglobin Method (Cat. No. 5334) for quantitation of HbA2, in the presence of HbS or the Helena Beta-Thal HbA2, QuikColumn Procedure (Cat. No. 5341) are recommended. HbA2 quantitation is one of the most important diagnostic investigations used in the evaluation of beta-thalassemia.

3. Low levels of HbF (1 to 10%) may be accurately quantitated by radial immunodiffusion using the Helena HbF-QuikPlate Procedure (Cat. No. 9352).

4. When a particular hemoglobin concentration varies significantly, 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 hemoglobins may be present, and a small amount of HbA2 and HbF is present. At the end of the first year of life and through adulthood, the major hemoglobin present is HbA with up to 3.5% HbA2, and less than 2% HbF.

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, thalassemias syndromes, life long cyanosis, hemolytic anemias or erythrocytosis or if the heterozygote is of sufficient prevalence for genetic counseling. The combinations of Hb S-S, Hb S-D, Los Angeles and Hb S-S-Arab lead to serious sickling disorders. Several variants including Hb H, H-E, S-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 complications 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 charac- terized by hypochromia and microcytosis due to the diminished synthesis of one or more globin chains (the u or d) while synthesis of the other chain proceeds normally.7,8 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 a-thalassemias the α-chains are diminished or absent, and in the β-thalassemia the β-chains are affected. Another qualitative 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 a normal development, are asymptomatic and have no anemia.9

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 migration positions, with HbA2 also present.

Sickle Cell Anemia

This is a homozygous state showing almost exclusively HbS, although a small amount of HbA2 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-Thalassemia HbA is absent.

Materials needed but not provided in the kit:

- ITEM
- CAT. NO.
- SPIFE 4000 Analyzer 1620
- Gel Block Remover 1621
- AFSC Hemo Control 1115
- SPIFE 4000 White Sample Trays 5331
- SPIFE 4000 Cassettes 1630
- SPIFE 4000 Maintenance Blotters 2307
- SPIFE 4000 Gel Staging Lid 2308

Materials needed but not provided:

- 5% acetic acid
- 0.85% saline

STEP BY STEP METHOD

I. SPIFE 4000 Preparation

1. Place SPIFE 4000 Applicator Blades (one per gel) beginning in the first position at the top of the Applicator Tray.

2. Fill the designated bottles with deionized water and destain.

3. Add prepared acid blue stain to the appropriate stain bottle.

4. The stain must be replaced after processing ten gels to avoid contamination.

5. Fill the DI Water Surfactant jar with deionized water and remove the lid and cover. Ensure that the ends of the tubing are below the water level.

6. Remove the antisept/water reservoir from the antisept station. Lift the cover, fill the reservoir and place back to the antisept station. Click the SPIFE 4000 icon on the screen for the instrument to initialize.
In Sickle Cell (*-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.

**BIBLIOGRAPHY**


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**SPIFE® 4000 Acid Hemoglobin Procedure**

**The SPIFE 4000 Acid Hemoglobin Electrophoresis Procedure** 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 function is 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 alpha-globin chains of 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 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 major hemoglobins most commonly seen in the United States are Hbs and Hbc. Hb Lepore, HbE, Hbg-Philadelphia, HbD-Los Angeles and HbE-Arab may be seen less frequently.

Electrophoresis is generally considered the best method for separating and identifying hemoglobinopathies. The protocol for hemoglobin electrophoresis involves step-wise use of two systems.

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 Procedure 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 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 packed.

---

**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.

**WARNING:** 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% 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.

**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 BYPY. Substitute the potassium cyanide.

**Preparation for Use:** The reagent is ready for use as packed.

**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% (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. 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 indicated on the package.

**Signs of Deterioration:** Discard if solution becomes cloudy.

**5. Destain Additive**

**Ingredients:** The product is a wetting agent.

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE ONLY. IRRITANT—DO NOT INGEST.

**Preparation for Use:** Pour the entire contents of the Destain additive bottle into the 1 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.

**Signs of Deterioration:** The additive should be free of precipitate.