2. After dissolution, the destain contains 0.3% (w/v) HbA, HbA2 and less than 2% HbF.

3. This is a homozygous state showing almost exclusively HbC.

4. C Disease

The SPIFE Acid Hemoglobin Electrophoresis Procedure is intended for the qualitative determination of hemoglobin using agar in acid buffer on the SPIFE, the SPIFE 2000 or the SPIFE 3000 system.

5. SUMMARY

Horizontal (HbA) is 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 hemoglobin chains, that are present in either the adult or fetal forms. A typical sequence of amino acids constitutes each of four polypeptide chains. Each polypeptide chain has two alpha (α) chains and two non-alpha chains. The alpha chains contain iron and the non-alpha chains do not.

6. Hemoglobinopathies

This is a homozygous state showing almost exclusively HbC.

7. 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.

8. Signs of Deterioration:

The diluted stain should be a clear, pale yellow solution.

9. Signs of Deterioration:

The reagent contains deionized water with 0.005 M EDTA, 150 mM NaCl, 50 mM potassium cyanide, 0.7% Triton X-100, and 0.1% sodium azide.

10. Hemolysate Reagent

The SPIFE Acid Hemoglobin Electrophoresis Procedure is intended for the qualitative determination of hemoglobin using agar in acid buffer on the SPIFE, the SPIFE 2000 or the SPIFE 3000 system.

11. All 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, and are of interest primarily to research scientists. Variants are clinically important when their presence leads to sickling disorders, and are of interest primarily to research scientists.

12. Signs of Deterioration:

The gels are ready for use as packaged.

13. Storage and Stability:

The reagents should be stored at room temperature (15 to 30°C) and must be used within one month of the expiration date indicated on the vial.

14. Signs of Deterioration:

The reagents should be stored at room temperature (15 to 30°C) and must be used within one month of the expiration date indicated on the vial.
I. Sample Preparation

1. Prepare lysates of patient specimens and controls as instructed in the "Specimen Preparation" section.

2. Remove one disposable Applicator Blade Assembly from the packaging. Remove the protective guard from the blade by gently bending the protective piece back and forth until it breaks free.

3. Place the Applicator Blade into the numbered vertical slots in the Applicator Assembly as given below:

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Slots</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPIFE 2000/3000</td>
<td>8</td>
</tr>
</tbody>
</table>

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.

4. If needed, place an Applicator Weight on top of the Applicator Blade.

5. If using SPIFE and the Auto Applicator, raise the Applicator Blades by flipping the toggle switch to the up position. Using the adjustment knob, set the Auto Applicator speed to 4.

6. Slide the Disposable Sample Cups strip into the center channel of the Cup Tray (numbered 1 to 20).

7. Pipette 17 µl of patient or control lysate into each of the Disposable Cups. Cover until ready for use.

II. Gel Preparation

1. Remove the gel from the protective packaging and discard the overlay.

2. Place a REP Blotter C on gel with the longer end parallel with the outside edge of each gel block outside the magnetic posts. Improper contact between the electrode and the gel blocks may cause skewed patterns. Close chamber lid.

3. Press the TEST SELECT/CONTINUE button to start electrophoresis. SPIFE will beep when electrophoresis is complete. Proceed to section IV.

B. SPIFE 2000

1. No Prompt
   - Load Sample 1
   - Load Sample 2

2. No Prompt
   - Apply Sample 1
   - Apply Sample 2

3. No Prompt
   - Electrolysis 1
   - Electrolysis 2

4. Remove gel blocks, (continue)
   - Dry 1
   - Dry 2

5. No prompt
   - END OF TEST

C. SPIFE 3000

1. No Prompt
   - Load Sample 1
   - Load Sample 2

2. No Prompt
   - Apply Sample 1
   - Apply Sample 2

3. No Prompt
   - Electrolysis 1
   - Electrolysis 2

4. Remove gel blocks, (continue)
   - Dry 1
   - Dry 2

5. No prompt
   - END OF TEST

IV. Visualization

1. After electrophoresis is complete, open the chamber lid and use the Gel Block Remover to remove the gel blocks. Place one electrode across each end of the gel to prevent curling during drying. Dispose of blades and cups as biohazardous waste.

2. Close the chamber lid and press the TEST SELECT/CONTINUE button to dry the gel.

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

4. Remove the Gel Holder from the stainer chamber. Attach the gel to the holder by placing the round hole over the left pin and the obround hole over the right pin on the holder.

5. Place the Gel Holder with the attached gel facing backwards into the stainer chamber.

6. Press the TEST SELECT/CONTINUE button until ACID HEMOGLOBIN appears on the display.

7. With ACID HEMOGLOBIN on the display, press the START/STOP button. An option to either begin the test or skip the operation will be presented. Press START/STOP to begin. The instrument will stain, destain and dry the gel.

8. When the gel has completed the process, the instrument will beep. Remove the Gel Holder from the stainer. Take the gel off of the holder.

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.

Quals 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 mark-er 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.
**I. Sample Preparation**

1. Prepare lysates of patient specimens and controls as instructed in the "Sample Preparation" section.

2. Remove one disposable Applicator Blade Assembly from the packaging. Remove the protective guard from the blade by gently bending the protective piece back and forth until it breaks free.

3. Place the Applicator Blade into the numbered vertical slots in the Applicator Assembly as given below.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Slots</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPIFE 3000</td>
<td>9</td>
</tr>
<tr>
<td>SPIFE 2000/3000</td>
<td>8</td>
</tr>
</tbody>
</table>

**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.

4. If needed, place an Applicator Weight on top of the Applicator Blade.

5. If using SPIFE and the Auto Applicator, press Auto Applicator Assembly onto the tray alignment pins. Low the Applicator Blades by flipping the toggle switch to the down position. Using the adjustment knob, set the Auto Applicator speed to 4.

6. Slide the Disposable Sample Cups strip into the center channel of the Cup Tray (numbered 1 to 20).

7. Pipette 17 µl of patient or control lysate into each of the Disposable Cups. Cover until ready for use.

**II. Gel Preparation**

1. Remove the gel from the protective packaging and discard the overlay.

2. Place the REP Blender C on the gel with the longer end parallel with the gel blocks. Gently blot the entire surface of the gel using light fingertip pressure on the blender, and remove the blotters.

3. Dispense approximately 2 mL of REP Prep onto left side of SPIFE chamber.

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 and ending on the right side, fitting the obround hole over the right pin. Use lint-free tissue to wipe around the edges of the gel backing, especially next to electrodes 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 edge of each gel block outside the magnetic posts. Improper contact between the electrode and gel blocks may cause skewed patterns. Close chamber lid.

7. Press the TEST SELECT/CONTINUE buttons located on the Electrophoresis and Stainer sides of the instrument until the ACID HEMOGLOBIN option appears on the displays.

**III. Application/Electrophoresis/Staining**

**Using the instructions provided in the appropriate Operator’s Manual, set up parameters as follows for the SPIFE, the SPIFE 2000, and the SPIFE 3000:**

### A. SPIFE Electrophoresis Unit

1. Load Sample 1: Place applicator on sample tray (continue)
2. Apply Sample 2: 00:30 20°C (Place applicator on chamber, continue)
3. Electrolysis 25.00 17°C 250 V0LT (Remove applicator, close lid, continue)
4. Dry: 3.00 54°C (Remove gel blocks, continue)
5. End of Test: No Prompt

#### Stainer Unit

<table>
<thead>
<tr>
<th>No Prompt</th>
<th>Stain</th>
<th>4.00</th>
<th>Recirculate OFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Prompt</td>
<td>1.00</td>
<td>Recirculate ON</td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>3.00</td>
<td>54°C</td>
<td></td>
</tr>
<tr>
<td>No Prompt</td>
<td>2.00</td>
<td>Recirculate ON</td>
<td></td>
</tr>
<tr>
<td>Destain</td>
<td>2.00</td>
<td>Recirculate ON</td>
<td></td>
</tr>
<tr>
<td>No Prompt</td>
<td>20.00</td>
<td>54°C</td>
<td></td>
</tr>
<tr>
<td>No Prompt</td>
<td>4.00</td>
<td>62°C</td>
<td></td>
</tr>
</tbody>
</table>

**IV. Visualization**

1. After electrophoresis is complete, open the chamber lid and use the Gel Block Remover to remove the gel blocks.

**B. SPIFE 2000 Electrolysis Unit**

1. Load Sample 1: 00:30 20°C SPD.+4
2. Apply Sample 1: 00:30 20°C SPD.+4
3. Electrolysis 1: 25.00 17°C 250 V0LT
4. Remove gel blocks, (continue)
5. No prompt

**C. SPIFE 3000 Electrolysis Unit**

1. Load Sample 1: 00:30 20°C SPD4
2. Apply Sample 1: 00:30 20°C SPD4 LOC 1
3. Electrolysis 1: 25.00 17°C 250V 85mA
4. Remove gel blocks, (continue)
5. End of TEST

#### Stainer Unit

<table>
<thead>
<tr>
<th>No Prompt</th>
<th>Stain 1</th>
<th>4.00</th>
<th>REC = OFF VALVE = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Prompt</td>
<td>Destain 1</td>
<td>1.00</td>
<td>REC = REV VALVE = 2</td>
</tr>
<tr>
<td>No Prompt</td>
<td>Destain 2</td>
<td>2.00</td>
<td>REC = REV VALVE = 2</td>
</tr>
<tr>
<td>No Prompt</td>
<td>Destain 3</td>
<td>2.00</td>
<td>REC = REV VALVE = 2</td>
</tr>
<tr>
<td>Dry</td>
<td>2.00</td>
<td>54°C</td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>2.00</td>
<td>62°C</td>
<td></td>
</tr>
</tbody>
</table>

6. **Remove the Gel Holder from the stainer chamber.**

7. **Press the TEST SELECT/CONTINUE button until ACID HEMOGLOBIN appears on the display.**

8. **When the gel has completed the process, the instrument will beep.** Remove the Gel Holder from the stainer. Take the gel off of the holder.

**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.

**Quartz 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.

**APPLICATION POINT**

- Aio Arab
- Akhirshar
- AEE
- 11.0% G-Hb
- AHI
- AIO San José
- AIO Thailand
- Ak-Punjabi
- Af-Doleman
- AIN
- AFSC
- AIO-keb
- Akhirshar
- AIO San José
- AIO-Punjabi
- AFSC
This is a homozygous state absolutely exclusively HbC. Thalassemia minor
This condition shows HbF and HbAα.

BIBLIOGRAPHY
3. Schneider, R.G., et al., Laboratory Identification of the Hemoglobins, Lab Manage-
ment, August, 29-41, 1981.
4. Center for Disease Control. Laboratory Methods for Detecting Hemoglobinopa-
5. Schneider, R.G., Methods for Detection of Hemoglobin Variants and Hemoglobin-
opathies in the Routine Clinical Laboratory, CRC Critical Reviews in Clinical/Lab-
orical Sciences, 1979.
11. Lehman, H. and Hunterman, R.G., Hereditary Spherocytosis. J.B. Lippincott Co., Phila-
delphia, 1974.

INTERPRETATION OF RESULTS

This condition shows HbA, HbF and HbC.

This is a heterozygous state demonstrating HbS and HbC.

Sickle Cell Trait
This is a heterozygous state showing almost exclusively HbS.

The haemoglobinopathies are a group of disorders charac-
terized by unbalanced synthesis of one or more globin chains. These precipitate within the red cell,
resulting in microcytic hypochromic red cells. These 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. tha-
lassemia)
3. Developmental anomalies (e.g. hereditary persistence of fetal hemoglobin (HPFH))

This method is based on the complex interactions of the hemoglo-
in, oxygen, pH, temperature, ions, and the presence of foreign substances.

The haemoglobinopathies are a group of disorders characterized by unbalanced synthesis of one or more globin chains. These precipitate within the red cell, resulting in microcytic hypochromic red cells. These 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. thalas-
assemia)
3. Developmental anomalies (e.g. hereditary persistence of fetal hemoglobin (HPFH))

This condition shows HbA, HbF and HbAα.

C Disease

This is a heterozygous state demonstrating HbS and HbC.

Sickle Cell-Thalassemia Disease
This condition shows HbA, HbF and HbAα. In Sickle Cell-Tα-Thalassemia HbAα is absent. In Sickle Cell-Tβ-Thalassemia HbAα is present in reduced quantities.

Thalassemia-C Disease
This condition shows HbA, HbAα, and HbAβ.

The thalassemias are a group of hemoglobin disorders charac-
terized by hypochromia and microcytosis due to the diminished
The thalassemias are a group of hemoglobin disorders characterized by hypochromia and microcytosis due to the diminished synthesis of one or more globin chains. These precipitate within the red cell, resulting in microcytic hypochromic red cells.

This condition shows HbA, HbF and HbAα.

Thalassemia-C Disease

This is a heterozygous state demonstrating HbS and HbC.

Sickle Cell-Tα-Thalassemia Disease
This condition shows HbA, HbF and HbAα. In Sickle Cell-Tα-Thalassemia HbAα is absent. In Sickle Cell-Tβ-Thalassemia HbAα is present in reduced quantities.

Thalassemia-C Disease
This condition shows HbA, HbAα, and HbAβ.

The thalassemias are a group of hemoglobin disorders characterized by hypochromia and microcytosis due to the diminished synthesis of one or more globin chains. These precipitate within the red cell, resulting in microcytic hypochromic red cells.

This condition shows HbA, HbF and HbAα.

Thalassemia-C Disease

This is a heterozygous state demonstrating HbS and HbC.

Sickle Cell-Tα-Thalassemia Disease
This condition shows HbA, HbF and HbAα. In Sickle Cell-Tα-Thalassemia HbAα is absent. In Sickle Cell-Tβ-Thalassemia HbAα is present in reduced quantities.

Thalassemia-C Disease
This condition shows HbA, HbAα, and HbAβ.

The thalassemias are a group of hemoglobin disorders characterized by hypochromia and microcytosis due to the diminished synthesis of one or more globin chains. These precipitate within the red cell, resulting in microcytic hypochromic red cells.

This condition shows HbA, HbF and HbAα.

Thalassemia-C Disease

This is a heterozygous state demonstrating HbS and HbC.

Sickle Cell-Tα-Thalassemia Disease
This condition shows HbA, HbF and HbAα. In Sickle Cell-Tα-Thalassemia HbAα is absent. In Sickle Cell-Tβ-Thalassemia HbAα is present in reduced quantities.

Thalassemia-C Disease
This condition shows HbA, HbAα, and HbAβ.

The thalassemias are a group of hemoglobin disorders characterized by hypochromia and microcytosis due to the diminished synthesis of one or more globin chains. These precipitate within the red cell, resulting in microcytic hypochromic red cells.

This condition shows HbA, HbF and HbAα.

Thalassemia-C Disease

This is a heterozygous state demonstrating HbS and HbC.

Sickle Cell-Tα-Thalassemia Disease
This condition shows HbA, HbF and HbAα. In Sickle Cell-Tα-Thalassemia HbAα is absent. In Sickle Cell-Tβ-Thalassemia HbAα is present in reduced quantities.

Thalassemia-C Disease
This condition shows HbA, HbAα, and HbAβ.

The thalassemias are a group of hemoglobin disorders characterized by hypochromia and microcytosis due to the diminished synthesis of one or more globin chains. These precipitate within the red cell, resulting in microcytic hypochromic red cells.

This condition shows HbA, HbF and HbAα.

Thalassemia-C Disease

This is a heterozygous state demonstrating HbS and HbC.

Sickle Cell-Tα-Thalassemia Disease
This condition shows HbA, HbF and HbAα. In Sickle Cell-Tα-Thalassemia HbAα is absent. In Sickle Cell-Tβ-Thalassemia HbAα is present in reduced quantities.

Thalassemia-C Disease
This condition shows HbA, HbAα, and HbAβ.

The thalassemias are a group of hemoglobin disorders characterized by hypochromia and microcytosis due to the diminished synthesis of one or more globin chains. These precipitate within the red cell, resulting in microcytic hypochromic red cells.

This condition shows HbA, HbF and HbAα.

Thalassemia-C Disease

This is a heterozygous state demonstrating HbS and HbC.

Sickle Cell-Tα-Thalassemia Disease
This condition shows HbA, HbF and HbAα. In Sickle Cell-Tα-Thalassemia HbAα is absent. In Sickle Cell-Tβ-Thalassemia HbAα is present in reduced quantities.

Thalassemia-C Disease
This condition shows HbA, HbAα, and HbAβ.

The thalassemias are a group of hemoglobin disorders characterized by hypochromia and microcytosis due to the diminished synthesis of one or more globin chains. These precipitate within the red cell, resulting in microcytic hypochromic red cells.