Sickle cell anemia (HbSS) is a cruel and lethal disease. It first manifests itself at about four months after birth which results in a continued high percentage of hemolysis. The major hemoglobin in the erythrocyte of the adult male is HbS and, in addition, newborns carry both HbS and HbF in large amounts. The HbF is progressively replaced by HbS. The hemoglobinopathies are of interest primarily to research scientists. Variants are clinically asymptomatic and have no anemia.

The determination of the mean percentage of HbA, HbS and HbC in a patient sample is an important tool in the diagnosis and management of thalassemia and sickle cell diseases.

HbA - 96.5% - 98.1%
HbS - 0.6% - 9.0%
HbC - 2.1% - 4.8%

A correlation study of the QuickGel Alkaline Hemoglobin and the SPIFE 2000 Analyzer 1130 was carried out. The SPIFE 2000 Analyzer 1130 is equipped with a cathode ray tube and an instantaneous X-RAY image detector. The system is capable of producing an X-RAY image of the sample for the detection of hemoglobinopathies.

The QuickGel Alkaline Hemoglobin electrophoresis procedure is used to determine the mean percentage of HbA, HbS and HbC in a patient sample using agarose electrophoresis in abut buffer or the SPIFE Alkaline Hemoglobin kit. The sample is run on a thin layer agarose gel and stained with Acid Blue stain. The percentage of HbA, HbS and HbC is determined using a 27 channel instrument. The results are used to screen for the presence of thalassemia, sickle cell disease and other hemoglobinopathies.

The QuickGel Alkaline Hemoglobin kit is used for the laboratory diagnosis of thalassemia, sickle cell disease and other hemoglobinopathies. The kit is used to determine the mean percentage of HbA, HbS and HbC in a patient sample. The percentage of HbA, HbS and HbC is determined using a 27 channel instrument. The results are used to screen for the presence of thalassemia, sickle cell disease and other hemoglobinopathies.

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To Two controls for hemoglobin electrophoresis are 2.

3.  Place the Applicator Blade into the verti

II. Sample Preparation

3.  Place the round hole in the guide over the left chamber pin and

1.  The SPIFE QuickGel Chamber Alignment Guide must be used

QuickGel Alkaline Hemoglobin Gels (10)

AFSC (Cat. No. 5331) 1:2 (1 part control + 1 part Hemolysate Reagent)

4.  Place an Applicator Weight on top of the Applicator Blade.

**Dry 2 = 5 to 8 minutes**

3.  Anion exchange column chromatography is a more accurate

Hemoglobin Gels (10)    Hemoglobin Gels (10)

Hemoglobin Gels (10)    Hemoglobin Gels (10)

Materials provided but not provided:

Materials needed but not provided:

**END OF TEST**

1) No Prompt

2) No Prompt

3) To Continue (continue)

7) No Prompt

4) No Prompt

2) No Prompt

1) No Prompt

2) No Prompt

3) No Prompt

1) No Prompt

3) No Prompt

**Dry 1 = 5 to 10 minutes**

9.  Destain the gel in two consecutive washes of Destain solution for

2.  Carefully place the template on the gel aligning the template

3.  Apply slight fingertip pressure to the template making sure

2.  Carefully place the template on the gel aligning the template

3.  Place the gel backing under one of the metal bars. Bend the gel backing

While holding the gel

6.  Place the gel into the stain for 4 minutes. Remove the gel

5.  After 30 seconds, lift the Applicator Knob and immediately

2.  With appropriate test name on the display, press the

3.  Place an Applicator Weight on top of the Applicator Blade.

9.  Destain the gel in two consecutive washes of Destain solution for

2.  Carefully place the template on the gel aligning the template

3.  Place the gel backing under one of the metal bars. Bend the gel backing

While holding the gel

1.  Remove one QuickGel Sample Template from the package.

2.  Place an Applicator Weight on top of the Applicator Blade.

9.  Destain the gel in two consecutive washes of Destain solution for

2.  Carefully place the template on the gel aligning the template

3.  Place the gel backing under one of the metal bars. Bend the gel backing

While holding the gel

6.  Place the gel into the stain for 4 minutes. Remove the gel

5.  After 30 seconds, lift the Applicator Knob and immediately

2.  With appropriate test name on the display, press the

3.  Place an Applicator Weight on top of the Applicator Blade.
I. Introduction

The dried gels are stable for an indefinite period of time. Prepare hemolysates of patient specimens and controls as instructed in the "Specimen Preparation" section.

II. Sample Preparation

A. SPIFE 2000/3000

1. Electrophorese the gel for *14:30 minutes at 550 Volts.

2. When the electrophoresis is complete, use the QuickGel Dispo Cup Tray (Cat. No. 1268) to collect the sample. Only use the Tray to collect samples, and do not mix the samples in it.

3. Place the gel in the chamber and close the Drying Lid.

B. QuickGel Blotter

4. Using a QuickGel Blotter C, gently blot the entire gel using the edges (not touching the gel) so that, when the lid closes, the edges of the blotter are imprinted on the gel, and start the power supply.

5. After the electrophoresis is complete, the operator must be able to see the results on the display. If not, the electrophoretic gel is not well set and must be removed from the chamber.

III. Gel Preparation

A. SPIFE Electrode

6. Place the electrode over the left chamber pin and secure it in place. Insert the other chamber pin into the Electrode and rotate it until it clicks.

7. When the gel is dry, remove the electrode and proceed with the next step.

B. QuickGel Gel Block Remover

8. Use the QuickGel Gel Block Remover to remove the gel from the chamber. Use a gentle twisting motion to free the gel from the chamber. When the gel is released, it should fall into the base.

IV. Electrophoresis/Staining

A. SPIFE 3000

9. Insert a chamber cover over the gel and the chamber.

10. Place the chamber cover on the base and secure it in place. The chamber cover is designed to keep the gel in its place and to prevent damage to the gel.

B. SPIFE 2000

11. Place the gel into the stain for 4 minutes. Remove the gel and let it drain for 1 minute before blotting.

C. QuickGel Blotter

12. Using the QuickGel Blotter, gently blot the entire gel with Destain Solution for 30 seconds. After blotting, the gel should be dry and ready for analysis.

V. Analysis

A. SPIFE Reader

13. Use the SPIFE Reader to view the gel. The gel is ready for viewing when the samples are visible on the display.

14. Use the QuickGel Chamber and dry for 10 minutes or until dry. When the gel is dry, the samples become visible on the display.

B. QuickGel Blotter

15. Using the QuickGel Blotter, gently blot the entire gel with Destain Solution for 30 seconds. After blotting, the gel should be dry and ready for analysis.

C. QuickGel Dispo Cup Tray

16. Place the QuickGel Dispo Cup Tray into the base. The tray is designed to collect the sample and to prevent damage to the gel.

VI. Evaluation of the Hemoglobin Bands

A. SPIFE 2000/3000

17. The bands seen on the gel must be separated by other methodologies. The Helena Beta-Thal HbA Method (Cat. No. 5334) is recommended. HbA and HbS must be differentiated by other methodologies.

B. QuickGel Blotter

18. The gel is ready for viewing when the samples are visible on the display. The samples must be differentiated by other methodologies.

C. QuickGel Dispo Cup Tray

19. The gel is ready for viewing when the samples are visible on the display. The samples must be differentiated by other methodologies.

VII. Stability of End Product

A. SPIFE 2000/3000

20. The dried gels are stable for an indefinite period of time. Prepare hemolysates of patient specimens and controls as instructed in the "Specimen Preparation" section.

B. QuickGel Blotter

21. The dried gels are stable for an indefinite period of time. Prepare hemolysates of patient specimens and controls as instructed in the "Specimen Preparation" section.

C. QuickGel Dispo Cup Tray

22. The dried gels are stable for an indefinite period of time. Prepare hemolysates of patient specimens and controls as instructed in the "Specimen Preparation" section.

VIII. Quality Control

A. SPIFE 2000/3000

23. Two controls for hemoglobin electrophoresis are recommended. HbA and HbS must be differentiated by other methodologies.

B. QuickGel Blotter

24. Two controls for hemoglobin electrophoresis are recommended. HbA and HbS must be differentiated by other methodologies.

C. QuickGel Dispo Cup Tray

25. Two controls for hemoglobin electrophoresis are recommended. HbA and HbS must be differentiated by other methodologies.

IX. Disposal

A. SPIFE 2000/3000

26. Use the QuickGel Chamber and dry for 10 minutes or until dry. When the gel is dry, the samples become visible on the display.

B. QuickGel Blotter

27. Use the QuickGel Chamber and dry for 10 minutes or until dry. When the gel is dry, the samples become visible on the display.

C. QuickGel Dispo Cup Tray

28. Use the QuickGel Chamber and dry for 10 minutes or until dry. When the gel is dry, the samples become visible on the display.

LIFEBRACKETS

In the Hemoglobin 2000/3000 method, the gel is stable for an indefinite period of time. Prepare hemolysates of patient specimens and controls as instructed in the "Specimen Preparation" section.

**Note:** The use of templates for sample application is offered as an alternative to the manual pipetting method. Instructions are provided for both methods.
The dried gels are stable for an indefinite period. Place the Applicator Blade into the verti-

II. Sample Preparation

I. Chamber Preparation

Materials needed but not provided:
- SPIFE 2000 Analyzer 1130
- QG-Blotter C (10)
- Hemolysate Reagent (25 mL)
- QuickGel Applicator Blades 1270
- SPIFE QuickGel Chamber Alignment Guide 86541003
- Hemolysate Reagent (25 mL) Citric Acid Destain (1 pkg)

PROCEDURE FOR SPIFE 2000/3000

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

2. Prepare the hemolysate solution. Mix the 10 µL sample to 150 µL Hemolysate Reagent.

3. Place the gel over the REP Prep inside the rectangle on the bottom of the electrophoresis chamber.

4. Then slowly lower the Applicator Knob allowing the blade to come into contact with the sheet. The gel is in contact with the electrodes and the gel block can result in skewed patterns.

5. At the end of the electrophoresis, the instrument will beep. When the process is complete, the instru-

6. Slide the Disposable Sample Cup strip into the row numbered 4 to 10. Cover the tray and discard the mold.

7. Place two Blotter X’s horizontally along the top and bottom sides of the gel backing. They should be positioned along the edges of the gel block and placed so that the blotters overlap the edge of the gel to be used from the plastic mold and discard the mold.

8. Place two Blotter X’s horizontally along the top and bottom

9. Close the lid, press the power switch to turn on the chamber

10. Destain the gel in two consecutive washes of Destain solution.

NOTE: A "Dry 1" time of 15 minutes and a "Dry 2" time of 7 minutes. Some abnormal hemoglobins have similar electrophoretic mobilities and are not detected. The use of templates for sample application is offered as an option. The hemoglobin bands are identified by the use of an electrically charged strip containing the correct hemoglobins. Some markers are available from Helena Laboratories: AA Anhydrase, Adult (Cat. No. 5328) no dilution is necessary

2. 15°C 10 minutes

3. Add 2 mL of Destain solution. Place the Cup Tray with samples on the Destainer. Close the cover.

4. The gel will then be placed into the Destainer for the specified Destain time.

5. Destain solution

6. Press the Destain button. An option to either begin the test or stop the Destainer will be presented. Press the DEST button. A Destain time of 3 minutes and a "Dry 2" time of 7 minutes

7. Destain solution

8. Use the QuickGel Blotter A to gently blot the excess sample from the gel. Dispose the blotter. Dispose of the waste as instructed in the "Specimen Preparation" of the

9. The gel background should be completely clear. Tap the gel to remove any excess moisture. Remove the gel from the metal bar. The two small notches in the backing must fit over the template and then apply fingertip pressure to the gel to the right side fitting the right notch to the right pin and blade by gently bending the protective

10. Place the gel into the horizontal Destain Wash area. The gel must be placed into the Destainer for the Destain time. The use of templates for sample application is offered as an option. The hemoglobin bands are identified by the use of an electrically charged strip containing the correct hemoglobins. Some markers are available from Helena Laboratories: AA Anhydrase, Adult (Cat. No. 5328) no dilution is necessary.

11. Destain solution

12. Place the Applicator Weight on top of the Applicator Blade.

13. Place the gel over the REP Prep inside the rectangle on the bottom of the electrophoresis chamber.

14. The gel will then be placed into the Destainer for the specified Destain time.

15. Destain solution

16. With appropriate test name on the display, press the START/CONTINUE button. An option to either begin the test or stop the Destainer will be presented. Press the TEST SELECT/CONTINUE button. A Destain time of 3 minutes and a "Dry 2" time of 7 minutes

17. Destain solution

18. The following materials needed for the procedure

19. The use of templates for sample application is offered as an option. The hemoglobin bands are identified by the use of an electrically charged strip containing the correct hemoglobins. Some markers are available from Helena Laboratories: AA Anhydrase, Adult (Cat. No. 5328) no dilution is necessary.

20. Destain solution

21. Destain solution
The gels should be stored horizontally at this condition shows HbF, HbA and HbA

Sickle Cell-Thalassemia Disease

This is a heterozygous state demonstrating HbS and HbC.

The thalassemias are a group of hemoglobin disorders characterized

In Sickle Cell

These values should only serve as guidelines. Each laboratory should

HbA  - 96.5% - 98.1%

Each of the two samples were run in replicate on four

Y = 1.004X - 0.169

they can be harmful. A drawback of this method is that many structurally different hemoglobins, the evaluation must be supple

This method is based on the complex interactions of the hemoglobin

The gels are ready for use as packaged.

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Washed packed cell hemolysates must be...