Hemoglobin Electrophoresis Procedure

**INTRODUCTION OF RESULTS**
Most hemoglobin variants cause no semblable 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 anemias or erythrocytosis, or if the heterozygote is of sufficient prevalence to warrant genetic counseling. The combinations of hemoglobin A, A2 and S, and Hb S-O Arab lead to serious sickling disorders. Several variants including Hb E, Hb F and Hb D may cause a thalassemic blood picture. The two variants hemoglobin of greatest importance in the U.S., in terms of frequency and pathology are Hb S and Hb C. Sickle-cell anemia (HbSS) is a cruel and potentially lethal disease. It first manifests itself at about 5-6 months of age. The clinical course presents amazing episodes of pain and temperature elevations with anemia, sillouettes, lethargy, and transit 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 in 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 hemolysis and microcytosis due to the diminished synthesis of one globin chain (the α or β) while synthesis of the other chain proceeds normally.1,2 These不平衡 synthesis results in unstable globin chains. These precipitate within the red cell, forming inclusion bodies that shorten the life span of the cell. In thalassemias, the α-chains are diminished or absent. In β-thalassemia, the β-chains are affected. Another quantitative disorder of hemoglobin synthesis, hereditary persistent fetal hemoglobin (HPFH), represents a genetic failure of the mechanism that turn off gamma chain synthesis in the fourth month after birth, which results in a continued high frequency 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.1,2

The most common abnormal hemoglobins are:

**Sickle Cell Trait**
This is a heterozygous state showing HbA and HbS, and a normal amount of HbF, on cellucleate assay. Results on chroate agar show hemoglobinins 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 HbF may also be present.

**Sickle-C Blood**
This is a heterozygous state demonstrating HbA, HbS, HbF, and HbA2. In Sickle-C, β-thalassemia HbA2 is absent.

**Sickle-C Disease**
This condition shows HbA, HbF and HbC.

**C Disease**
This shows HbC as the only abnormal hemoglobin.

**β-thalassemia Major**
This condition shows HbF, HbA2 and HbC.

**BIBLIOGRAPHY**
11. Personal communication from Dr. Virgil Fairbanks.

For Sales and Order Information, and Technical or Service Assistance, call 800-231-5683 first.

Helena's Hemoglobin Electrophoresis Procedure, using cellucleate analysis, provides the quick and qualitative quantitative determination of abnormal hemoglobins.

**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 opposite direction. They are composed of two alpha and two beta globin chains. HbC is a well-studied normal adult hemoglobin (HbA), the non-alpha chains are called beta. The non-alpha chains of fetal hemoglobin are called gamma. A minor (3%) hemoglobin fraction called HbE consists of two alpha and one non-alpha chain. This condition shows HbA and HbE. In an identical state called thalassemia, the non-alpha chains may aggregate to form HbS (S-trypsin) or Hb Bart's (β4).

The major hemoglobin in the erythrocytes of the normal adult is HbA and its minor variants. The non-alpha chains of HbA are called delta chains. In a hereditary inhibition of globin chain synthesis called thalassemia, the non-alpha chains may aggregate to form HbH (HbA2α2δ2) or Hb Bart's (β4).

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

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST. HARMFUL IF SWALLOWED.

Preparation for Use:
1. Dissolve one vial of stain in 1 L deionized water.
2. Storage and Stability: The stain should be stored at 15 to 30°C and is stable until the expiration date indicated on the bottle.

**BIBLIOGRAPHY**
1. PermaClear® Solution (Cat. No. 4950) - Optional Ingredients: N-methyl pyrrolidinone and PEG.

**PRINCIPLE**
Electrophoresis is generally considered the best method for separating and identifying hemoglobinopathies. One protocol for hemoglobin electrophoresis involves the use of two systems.8-10 Initial electrophoresis is performed in alkaline buffers. Cellulose acetate is the major support material. In this condition, the HbF is blocked and many other variants with minimal preparation time. However, because the electrophoretic separation by similarity of many structurally different hemoglobinins, the evaluation must be supplemented by a procedure that measures the size of the hemoglobin molecules. The size of every hemoglobin can be measured relative to the complex interactions of the hemoglobin with the electrophoretic buffer. Electrophoresis is a simple procedure requiring only minutes quantities of hemolysate to provide highly specific (but not absolute) confirmation of the presence of HbF, HbC, HbS and HbA2 as well as several other abnormal hemoglobins.

**PRINCIPLES**
Very small samples of hemolysates prepared from whole blood are applied to the Titit® Cellulose Acetate Plate. The hemoglobins in the sample are separated on cellulose acetate by an alkaline buffer (pH 8.2-8.6), and are stained with PermaClear Stain. The patterns are scanned with a scanning densitometer, and the relative percent of each band determined.

**REAGENTS**
1. Supre-Heme® Buffer (Cat. No. 5802)
   - Ingredients: The buffer contains Tris-EDTA and boric acid. WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. NEVER PIPETTE BY MOUTH. Do not ingest. Ingestion of sufficient quantities of boric acid and EDTA can be toxic.
   - Preparation for Use: Dissolve one package of buffer in 980 mL deionized water. The buffer is ready for use when all material is dissolved and completely mixed.
   - Storage and Stability: The packaged buffer should be stored at 15 to 30°C and is stable until the expiration date indicated on the package and box. The buffer solution is stable two months when stored at 15 to 30°C.
   - Signs of Deterioration: Do not use packaged buffer if the material shows signs of discoloration or discoloration. Discard the buffer solution if it shows signs of bacterial contamination.

2. PermaClear Solution (Cat. No. 4950)
   - Ingredients: The reagent contains 0.005 M EDTA in deionized water with methanol added as a preservative. WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT PIPETTE BY MOUTH. The reagent contains a small amount of potassium cyanide.
   - Preparation for Use: The reagent is ready to use as packaged. Store the solution at 15 to 30°C and is stable until the expiration date indicated on the container. It may be stored in the bottle or in a tightly closed staining dish and may be reused multiple times if properly stored.
   - Signs of Deterioration: Do not use the stain solution if excessive evaporation occurs, or if large amounts of precipitate appear.

3. Clear Aid® (Cat. No. 5005)
   - Ingredients: The reagent contains polyethylene glycol. WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST.
   - Preparation for Use: Clear Aid is used as the clearing solution which is prepared as follows: 30 parts glacial acetic acid 70 parts absolute methanol 4 parts Clear Aid
   - Storage and Stability: Store the prepared clearing solution at 15 to 30°C in a tightly closed container to prevent evaporation of the methanol. When evaporation occurs, the plate may delaminate. Water contamination from over-use of the clearing solution will cause the clearing solution to turn cloudy. Water contamination can be prevented by storing the clearing solution as indicated on the bottles.
   - Signs of Deterioration: Clear Aid should be clear, colorless liquid, although it may appear cloudy when cold. Do not use the material upon evidence of gross contamination or discoloration. Discard the Clear Aid if this occurs before the clearing procedure.

4. PermaClear® Solution (Cat. No. 4950) - Optional
   - Ingredients: N-methyl pyrrolidinone and PEG.

5. PermaClear® Solution (Cat. No. 4950) - Optional
   - Ingredients: N-methyl pyrrolidinone and PEG.

6. **Instruments**
   - Any high quality scanning densitometer capable of scanning a cleared cellulose acetate plate at 254 nm may be used. Recommended is the Helena EDC (Cat. No. 1387), ClinScan® 3 (Cat. No. 1680) or other Helena instruments.

7. **SPECIMEN COLLECTION AND HANDLING**
   - Specimen: Whole blood collected in tubes containing EDTA or heparin is the specimen of choice.
   - Specimen Preparation: Specimen hemolysates are prepared as outlined in the STEP-BY-STEP METHOD.
Specimen Storage and Stability: Whole blood samples may be stored up to one week at 2 to 8°C.

PROCEDURE
Materials Provided: The following materials needed for the procedure are available from Helena Laboratories.

Hardware
- Super Z-12 Aplicator Kit (12 samples) 5023
- Super Z Aplicator Kit (8 samples) 4088
- Zip Zone Chamber 2838
- Microdispenser and Tubes 6008
- 1000 Staining Stil 5122
- Bufferz 5093
- I.O.D. 1126
- Titan Plus Power Supply 1504

Consumables
- Titan® III Cellulose Acetate (94 mm x 76 mm)-12 samples 3021
- Titan® Cellulose Acetate (76 mm x 60 mm)-8 samples 5330
- Hemo AFS Control 5328
- Hemo AC Control 5329
- Hemo AFSC Control 5331
- Hemo A5A Control 5329
- Hemolyse Reagent 5125
- Ponceau S 5526
- Clear Aid 5050
- Titan Blotter Pads 5034
- Zip Zone® Prep 5502
- Titan Plastic Envelopes 5052
- Helena Marker 5000
- Identification Labels 5006
- Zip Zone® chamber Wicks 5081
- Glue Stick 5002
- PemaGlue 4950

Materials Needed, but not Provided:
- Glacial acetic acid
- Absolute methanol
- 5% acetic acid – Mix 5 parts of glacial acetic acid with 95 parts deionized water.

SUMMARY OF CONDITIONS
- Plate..................................................Titan® III-H
- Supre-Heme® dissolved in 980 mL deionized water
- Soaking Time for Plates...................................5 minutes
- Temperature.................................................25°C
- Number of Applications...........................................1
- Electrolyte Time..................................................5 minutes
- Scanning Wavelength...........................................525 nm
- Soaking Time....................................................5 minutes
- Drying Time....................................................10 minutes at 95°C
- Scanning Wavelength...........................................525 nm

STEP BY STEP METHOD
A. Preparation of the Titan® III-H Plate
1. Dissolve one package Supre-Heme® Buffer in 980 mL deionized water.
2. Properly code the required number of Titan® III-H Plates by marking on the glossy hard side with a marker.
3. Soak the required number of plates in Supre-Heme® Buffer for 5 minutes. The plates should be soaked in the buffer according to the instructions provided. Alternatively, the plates may be wetted by slowly and unevenly pressing a rack of plates into the buffer.
4. The same soaking buffer may be used for soaking up to 12 plates or for approximately one week if stored tightly closed. If used for a prolonged period, residual solvents from the plates may build up in the buffer and cause poor separation of the proteins, or evaporation may cause greater buffer concentration.

B. Preparation of Zip Zone® Chamber
1. Pour approximately 100 mL of Supre-Heme® Buffer into each of the outer sections of the Zip Zone® Chamber.
2. Wet two chamber wicks in the buffer (Figure 1). Place the wicks over each support bridge being sure it makes contact with the buffer and that there are no air bubbles under the wicks.
3. Cover the chamber to prevent buffer evaporation. Discard the buffer and wicks after use.

Alternative Sample Preparation Procedure:
If removal of denatured hemoglobin from the sample is deemed necessary, perform the following:
1. Centrifuge the blood sample at 3500 RPM for 5 minutes.
2. Remove the clots from the sample and wash the red blood cells in 0.85% saline (v/v) three times. After each wash, centrifuge the cells at 1000 RPM for 30 minutes.
3. Add 1 volume deionized water and 1/4 volume toluene (or carbon tetrachloride) to the washed red cells. Vortex at high speed for one minute. Centrifuge the samples at 3500 RPM for 10 minutes.
4. If toluene is used, the top layer in the tube will contain cell stroma and should be removed with a capillary pipette before proceeding to the next step. If carbon tetrachloride is used, all red cell waste material will be contained in the bottom of the tube and should be discarded.
5. Discard the red solution through two layers of Whatman #1 filter paper.

D. Electrophoresis of Sample Plate
1. Quickly place the plate in the electrophoresis chamber, cellulose acetate side up. Place the electrode (electrically positive) toward the cathode (-) side of the chamber. Place a weight (glass slide, etc.) on the plate to insure contact with the wells.
2. Place the cover on the chamber, and electrophorese the plate for 20 minutes at 350 volts.

E. Staining the Hemoglobin Bands
1. Remove the plate from the electrophoresis chamber and stain in Ponceau S for 5 minutes.
2. Drain any excess solution. Then place the plate, acetate side up, onto a blotted paper, and into a 1:50 Micro-Hoo, or other drying oven at 50-60°C for 15 minutes or until dry.

If using a Clear Aid solution:
1. Drench, by washing the plate twice in absolute methanol, for two minutes each wash. Allow the plate to drain for 5-10 seconds before placing in the next solution.
2. Place the plate into the Clear Aid solution for 5-10 minutes.
3. Drain off excess solution. Then place the plate, acetate side up, onto a blotted paper, and into an I.O.D., Micro-Hood, or other drying oven at 50-60°C for 15 minutes or until dry.

If using a Ponceau S solution:
1. Place the plate(s) into the diluted PonceaClear cleaning solution for 2 minutes.
2. Drain off excess solution by holding plate(s) vertically for 1 minute. Then place the plate, acetate side up, onto a blotted paper, and into an I.O.D., or other drying oven at 50-60°C for 15 minutes or until dry.

F. Evaluation of the Hemoglobin Bands
1. Qualitative evaluation: The hemoglobin bands may be inspected visually for the presence of abnormal hemoglobin bands. The Helena Hemo Controls provide a marker for band identification.
2. Quantitative evaluation: The ratio of each hemoglobin band to the total hemoglobin band is determined by scanning the cleared plates and in the plates stained with a Ponceau S solution.

Stability of End Product: The dried plates are stable for an indefinite period of time and may be stored in Titan Plastic Envelopes.

Calculation: Calibration is a curve that is necessary because relative concentrations of the bands is the only parameter determined.

Quality Control: Four controls for hemoglobin electrophoresis are available from Helena Laboratories: (a) Hemo Control (Cat. No. 5330), (b) Hemo A5A Control (Cat. No. 5331), and (c) AFSC Hemo Control (Cat. No. 5332). The controls should be used to establish banding patterns. The values may be converted to percentage values by using the calibration curves.

Calculation of Unknown: The Helena EDC, ClinScan 3 and other Helena equipment can be used with these controls automatically to print the relative percent of the bands.

RESULTS
Figures 1 illustrates how the combination of cellulose acetate and citrate agar electrophoresis can be used in tandem for the identification of hemoglobin variants. Figure 2 lists the relative mobilities of various hemoglobin variants on cellulose acetate and citrate agar gels.

LIMITATIONS
Some variants of hemoglobin have similar electrophoretic mobilities and must be differentiated by other methodologies.

FURTHER INFORMATION
1. Citrate agar electrophoresis may be a necessary follow-up test for confirming the identity of variants detected on cellulose acetate.
2. Isoelectric focusing, high-performance liquid chromatography, gel electrophoresis, mass spectrometry (both acid and alkaline) and structural studies may be necessary in order to positively identify some of the rare hemoglobins.
3. The exchange column chromatography is the most accurate method for quantifying HbA2. Helena Laboratories’ Stickle-Thal Quick Column is offered for a separation of HbS, HbA2, and beta-Thal. The Quick Column® Procedure (Cat. No. 5341) is recommended. HbF quantitation is one of the most important diagnostic tests in the diagnosis of thalassemia trait.

4. Low levels of HbF (1-10%) may be accurately quantitated by radial immunodiffusion using the Helena Hemo-QUIF Plate Procedure (Cat. No. 9325).

REFERENCE VALUES
HbA2: Migration varied in citrate agar from hemoglobin 6 through Hemoglobin C. Normal range is 1-2%.
HbF: Tk cell (Tk) is approximately 20% of total.
Hbs: Hemoglobin S, D, and E.

*Los Angeles and D Punjab are the same hemoglobin.

World Health Organization is the same hemoglobin.

HbS is broadly identical on both media due to instability.

Figure 1. Electrophoretic Mobilities of Hemoglobins on Titan® III Cellulose Acetate and on Titan® IV Citrate Agar.

Figure 2. Relative Electrophoretic Mobilities of Hemoglobins on Cellulose Acetate and Citrate Agar.
**PROCEDURE**

Materials Provided: The following materials needed for the procedure are available from Helena Laboratories:

- **Hardware**
  - Cat. No. 5033
  - Super Z-12 Applicator Kit (12 samples)
  - Super Z Applicator Kit (8 samples)
  - Super Z Tube
  - Zip Zone® Chamber
  - Microdispenser and Tubes
  - 1000 Staining Set
  - Bufferzer
  - LO.D.
  - Titan Plus Power Supply

- **Consumables**
  - Titan II® Cellulose Acetate (94 mm x 76 mm) - 12 samples
  - Titan II® Cellulose Acetate (76 mm x 60 mm) - 8 samples
  - Supre-Heme® Buffer
  - Hemo AFSA® Control
  - Hemo AA2® Control
  - Hemo AFSC® Control
  - Hemo AlSa® Control
  - Hemolysate Reactant
  - Ponceau S
  - Clear Aid
  - Hemo AFS Control
  - Zip Zone® Prep
  - Titan Plastic Envelopes
  - Markers
  - Identification Labels
  - Zip Zone® chamber Wicks
  - Glue Sticks
  - PemaCrd

**Materials Needed, but not Provided:**
- Glacial acetic acid
- Absolute methanol
- 5% acetic acid – Mix 5 parts of glacial acetic acid with 95 parts deionized water.

**SUMMARY OF CONDITIONS**

- **Plate**
  - Titan II®

- **Supre-Heme® dissolved in 980 mL deionized water**

- **Soaking Time for Plates**
  - 5 minutes

- **Temperature**
  - 25 ± 0.05°C

- **Drying Time**
  - 10 minutes at 85°C

- **Scanning Wave length**
  - 525 nm

**STEP BY STEP METHOD**

A. Preparation of the Titan® II® Plate

1. Dissolve one package Supre-Heme® Buffer in 980 mL deionized water.

2. Properly code the required number of Titan™ II® Plates by marking on the glossy hard side with a marker.

3. Soak the required number of plates in Supre-Heme® Buffer for 5 hours.

4. The plates should be soaked in the buffer at least 5 hours.

5. The plates may be wetted by slowly and uniformly pouring a sack of plates into the buffer.

6. The same soaking buffer may be used for soaking up to 12 plates or for approximately one week if stored tightly closed. If used for a prolonged period, residual soaps from the plates may build up in the buffer and cause poor separation or evaporation, which may cause greater buffer concentration.

B. Preparation of Zip Zone® Chamber

1. Pour approximately 100 mL of Supre-Heme® Buffer into each of the outer sections of the Zip Zone® Chamber.

2. Wet two chamber wicks in the buffer. Allow the wicks to rise over each support bridge being sure it makes contact with the buffer and that there are no air bubbles under the wicks.

3. Cover the chamber to prevent buffer evaporation. Discard the buffer and wicks after use.

C. Sample Preparation and Application

1. Prepare a hemolysate of the patient samples as follows:
   - a. Using whole blood: Add 1 part whole blood to 3 parts Helena Reagent, Mix well and allow to stand for 5 minutes.
   - b. Using packed cells: Mix 1 part packed red blood cells to 6 parts Helena Reagent, Mix well and allow to stand for 5 minutes.

2. The plate is full, the major hemoglobin present is HbA with up to 3.5% HbA2.

3. To prevent evaporation, cover the Sample Well Plates using the Microdispenser. Do not prepare a hemolysate for the Reference Samples.

4. Prise the applicator by depressing the tips into the sample wells 3 or 4 times. Apply this loading to a piece of blotter paper. Priming the applicator makes the second loading much more uniform. Do not load applicator again at this point, but proceed quickly to the next step.

5. Remove the wetted Titan™ III Plate from the buffer with the fingertips and blot firmly between two blotter. Place the plate in the Helena EDC, CliniScan Cellulose Acetate, aligning the top edge of the plate with the black strap line marked CATHODE APPLICATION. The identical mark should be aligned with sample No. 1. Buffer facing the plate in the aligning base, drop a plate of sample solution onto the aligning base, but may be removed from the aligning base. This prevents the plate from bending during the sample application.

6. Apply the samples to the plate by depressing the applicator tips into the sample wells 3 or 4 times. Remove the applicator from the aligning base, and load the plate(s) down and hold it 5 seconds.

Alternative Sample Preparation Procedure:

If removal of denatured hemoglobins from the sample is deemed necessary, perform the following:

1. Centrifuge the blood sample at 3500 RPM for 5 minutes.

2. Remove the clots from the sample and wash the red blood cells in 0.85% saline (v/v) three times. After each wash, centrifuge the cells at 10,000 rpm for 10 minutes.

3. A 1 volume deionized water and 1/4 volume tolune (or carbolic acid base) to the washed red cells. Vertix at high speed for one minute. Centrifuge the samples at 3500 RPM forPath 4 minutes and throw away the supernatant.

4. If tolune is used, the top layer in the tube will contain cell stroma and should be removed with a capillary pipette before proceeding to the next step. Clear middle layer contains desired sample. If carbon tetrachloride is used, all red cell washes should be discarded. Vertix at high speed for one minute. Centrifuge the samples at 3500 RPM for 4 minutes and discard the supernatant.

5. If tolune is used, the top layer in the tube will contain cell stroma and should be removed with a capillary pipette before proceeding to the next step. Clear middle layer contains desired sample. If carbon tetrachloride is used, all red cell washes should be discarded. Vertix at high speed for one minute. Centrifuge the samples at 3500 RPM for 4 minutes and discard the supernatant.

D. Electrophoresis of Sample Plate

1. Quickly place the plate in the electrophoresis chamber, cellulose acetate side up, and run for 30 minutes.

2. Stop the electrophoreses when the cathode is at the bottom of the plate.

3. Place the cover on the chamber, and electrophorize the plate for 25 minutes at 350 volts.

E. Staining the Hemoglobin Bands

1. Remove the plates from the electrophoresis chamber and stain in Ponceau S for 5 minutes.

2. Distinguish in 3 successive washes of 5% acetic acid. Allow the plates to stay in each wash 2 minutes until the background is clear.

3. The plates may be dried and stored for a permanent record at this point. If a transparent background is desired for densitometric analysis, proceed to the next step.

F. Using Clear Aid Solution

1. Dehydrate, by washing the plate twice in absolute methanol, for two minutes. Allow the plate to drain for 5-10 seconds before placing in the next solution.

2. Place the plate into the Clear Aid solution for 5-10 minutes.

3. Drain off excess solution. Then place the applicator side up onto a blotter, and into an I.O.D., Micro-Hood, or other dryer oven at 50-60°C for 15 minutes or until dry.

4. Using Clear Aid Solution

5. Place the plate(s) into the dilitated Pemaclear cleaning solution for 2 minutes.

6. Drain off excess solution by holding plate(s) vertically for 1 minute. Then place the plate, acetate side up, onto a blotter and into an I.O.D., or other drying oven at 50-60°C for 15 minutes or until dry.

F. Evaluation of the Hemoglobin Bands

1. The dried plate may be visually examined for the presence of abnormal hemoglobin bands. The Helena Hemo Controls provide a marker for band identification.

2. The Helena Hemo Controls can be used to evaluate the relative amount of each hemoglobin band scanned by the clearing and dried plates and in the plates scanned by cellulose acetate. A calibration curve is necessary because relative concentration of the plate is the only parameter determined.

3. Quality Control: Four controls for hemoglobin electrophoresis are available from Helena Laboratories, AA2 Hemo Control (Cat. No. 5328), AlSa, Hemo Control (Cat. No. 5330), and APSA, Hemo Control (Cat. No. 5331). The controls should be used at times and properly transfixed that the hemoglobin bands, and they may be quantified for verification of the accuracy of the procedure. The package insert provided with the controls for assay values and migration patterns. Use at least one of these controls on each plate.

**RESULTS**

**Figure 1** illustrates how the combination of cellulose acetate and citrate agar electrophoresis can be used in tandem for the identification of abnormal hemoglobins. Figure 2 lists the relative mobilities of various hemoglobin mutants on cellulose acetate and citrate agar plates.

**Calculation of Unknown:**

1. The Helena EDC, CliniScan Comparison with these controls will automatically print the relative percentage of the bands.

**LIMITATIONS**

Some abnormal hemoglobins have similar electrophoretic mobilities and must be differentiated by other methodologies.

F. Evaluation of the Hemoglobin Bands

1. Citrate agar electrophoresis may be a necessary follow-up test for confirmation of abnormal hemoglobins detected on cellulose acetate.

2. Isoelectric focusing, high performance liquid chromatography, globin chain analysis and (both acid and alkaline) and structural studies are necessary in order to positively identify some of the rare hemoglobin.

3. An exchange column chromatography is the most accurate method for quantitating hemoglobins. Helena Laboratories’ Sickle-Thal Quick Column is the material to be used in the column of the tube water centrifugation.

4. The results of a hemoglobin is one of the most important diagnostic tests in the diagnosis of β-thalassemia trait.

**REFERENCE VALUES**

Quantiﬁcation of hemoglobin in the erythrocytes of the normal individual is total hemoglobin. HbF. Some of the major adult hemoglobin, HbA, and HbA2. But the minor using a S+4, are also present. At this stage, 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.
Hemoglobin Electrophoresis Procedure

**SUMMARY**

Hemoglobin (Hb) is a group of proteins whose chief functions are to transport oxygen from the lungs to the tissues and carbon dioxide in the opposite direction. They can be divided into the family of normal alpha chains, called globin, and iron protoporphyrin heme groups. A specific sequence of amino acids forms the globin chains of four possible primary structures. Each normal adult hemoglobin molecule contains one pair of alpha and one pair of non-alpha chains. The non-alpha chains are called beta. The non-alpha chains of fetal hemoglobin are called gamma. A minor (3%) globin fraction called HbC, contains alpha and gamma chains. This is a homogenous state, that in conjunction with the non-alpha chains may aggregate to form HbH (or 4 beta). The major hemoglobin in the erythrocytes of the normal adult is HbA and contains an equal amount of HbA2. In addition, 400 mutant hemoglobins are now known, some of which may cause serious or life-threatening conditions. One state or in combination with another abnormal hemoglobin. 4 Electrophoresis divides the abnormal forms of hemoglobin 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).

Hemoglobin abnormalities are classified in one of two ways in order to provide a guide for the clinician: by phenotype or by disease. Hemoglobinopathies are commonly classified into two categories: 1) abnormal variants, or 2) disease states.

**BIBLIOGRAPHY**

11. Personal communication from Dr. Virgil Fairbanks.

Hemoglobin Electrophoresis Procedure, using cellulose acetate as the supporting medium, has become the most widely used method for the qualitative and quantitative determination of abnormal hemoglobins.

**SUMMARY**

Hemoglobin (Hb) is a group of proteins whose chief functions are to transport oxygen from the lungs to the tissues and carbon dioxide in the opposite direction. They can be divided into the family of normal alpha chains, called globin, and iron protoporphyrin heme groups. A specific sequence of amino acids forms the globin chains of four possible primary structures. Each normal adult hemoglobin molecule contains one pair of alpha and one pair of non-alpha chains. The non-alpha chains are called beta. The non-alpha chains of fetal hemoglobin are called gamma. A minor (3%) globin fraction called HbC, contains alpha and gamma chains. This is a homogenous state, that in conjunction with the non-alpha chains may aggregate to form HbH (or 4 beta). The major hemoglobin in the erythrocytes of the normal adult is HbA and contains an equal amount of HbA2. In addition, 400 mutant hemoglobins are now known, some of which may cause serious or life-threatening conditions. One state or in combination with another abnormal hemoglobin. Electrophoresis divides the abnormal forms of hemoglobin 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).

Hemoglobin abnormalities are classified in one of two ways in order to provide a guide for the clinician: by phenotype or by disease. Hemoglobinopathies are commonly classified into two categories: 1) abnormal variants, or 2) disease states.