I. PRINCIPLE:

Cellulose acetate electrophoresis should be used for screening abnormal hemoglobins, including sickle hemoglobin, in anyone over 3 months of age.

Hemoglobins are composed of 2 pairs of globin chains with each chain consisting of amino acids in linear sequence. Some of the amino acids have hydrophilic side chains and therefore may be positively or negatively charged depending on the pH of the medium. Hemoglobin electrophoresis is based on the differing rates of migration of these charged molecules in an electric field. Temperature, pH, voltage, ionic strength of buffer and supporting medium all affect the migration pattern.

The distance between Hb A and its variants depends on the net charge of the molecules. On cellulose acetate at pH 8.6, S and C hemoglobins migrate more slowly toward the anode than Hg A. Hbs H, I, N and J migrate more rapidly toward the anode than A because they are more negatively charged.

A hemolysate is made and applied to cellulose-acetate. The cellulose-acetate is placed in the electrophoresis chamber for 25 min. and stained with Ponceau S.

II. SPECIMEN:

EDTA or heparinized blood, refrigerated until use.

III. REAGENTS:

A. Supre-Heme Buffer – 14.6 g Tris – EDTA – Boric Acid Buffer pH 8.2 – 8.6; 1 packet diluted to 1 liter with distilled water. Helena Laboratories. Store at 15° - 30°C. Stable for 2 months.

B. Hemolysate Reagent - 0.005 M EDTA, H_2O and hemoglobin preservatives; store at room temp.

C. Ponceau S stain, prepare according to manufacturer's instructions, expiration date as indicated by manufacturer.

D. 5% acetic acid: 50ml Glacial Acetic Acid in 950 ml distilled water. Stable for 1 year at room temperature.

E. Control hemolysate: AFSC Hemoglobin control, Helena Lab.

F. Normal (AA) blood from previous HPLC run.

IV. EQUIPMENT:

A. Electrophoresis cell designed for room temperature electrophoresis - Helena zip zone chamber.

B. Paper wicks

C. Cellulose acetate plates - Titan III-H, Helena Laboratories.

D. Super Z sample applicator

E. Helena welled sample holder - Zip zone sampler well plate.

F. Helena aligning base for cellulose acetate plates.

V. QUALITY CONTROL:

On each cellulose acetate sheet a known control containing AFSC and a normal patient with hemoglobin AA from previous HPLC must be run. These known bands will be used to identify the bands in the patients' electrophoresis patterns.

VI. PROCEDURE:

A. Allow EDTA samples to settle so there is a distinct RBC/plasma layer or spin at 3500 rpm for 5 min. Remove the AFSC standard from the refrigerator.

B. Soak cellulose acetate sheets in buffer for at least 5 min. adding them to the buffer slowly to avoid formation of air bubbles.

C. Add buffer to wells at either side of the zip zone chamber making sure the electrode wires are covered and the center well is dry.

D. Apply paper wicks to inside well walls by wrapping them up over walls making sure that they will be kept continuously wet during the procedure.

E. Label 12 X 75 tubes for each sample and dispense 0.5 ml of hemolysate reagent to each tube. (CAP samples 50 µl of sample with 150µl of hemolysate reagent.)

F. Add 90 µl from the red cell layer to the hemolysate reagent.

G. Vortex and allow to sit for 5 min.

H. Place standards and patient hemolysates in the zip zone sample well plate using 10 µl of sample. Write down the order of patient and control samples on the acetate.

I. Remove cellulose paper from buffer dish, blot with blotting paper.

J. Place paper in aligning base with the edge of the paper at the ***cathode*** line.

K. Prime applicator and apply samples to cellulose paper.

L. Place acetate paper on wicks face down bridging center well and replace cover on the chamber.

L. Connect the positive and negative leads of the electrophoresis chamber to the plugs.

Turn on the power switch. A beep will sound and the instrument will run a self-check. After approximately 2 seconds, dashes will appear on the displays indicating that the power supply is read for use. If a code appears instead, refer to the troubleshooting table in the instrument instruction book.

Press VOLTS, enter 350.00 using the keypad. When the correct voltage is displayed, press mA.

The mA (current) is 4.00 enter this number using the key pad. When the correct current is displayed, press TIME.
The Time display will **always** show 10 min 00 sec.
Change the time to 25 minutes 00 seconds using the keypad.

Using a timer let the acetate paper equilibrate for at least 5 minutes before starting electrophoresis. This is necessary to ensure complete electrical contact. If an error code (E04) appears, allow plates to equilibrate a few more minutes. Then press RUN/WAIT to

start electrophoresis. At the end of the run, a beep will sound and power will be turned off. The displays will return to dashes only.

To bypass the timer for any reason, simply press the RUN/WAIT key a second time. The timer will stop but power will remain on. To resume timing, press RUN/WAIT a third time (or press EXIT to stop the run).

Voltage = 350, Current (mA)= 4, Time = 25 minutes

M. After 25 minutes, remove cellulose paper and stain in Ponceau S for 2 minutes.

O. Destain in 5% acetic acid until background clears (approximately 3 washes of 2-5 minutes each, actively rocking to accelerate clearing)

P. Dry cellulose paper quickly by placing in a rack before a fan set at low speed or air dry if time permits. When completely dry place in a plastic envelope.

Q. Label each location with appropriate patient name, accession number, Control lot # and exp. date, or AA specimen designation.

R. Save hemolysates for citrate agar electrophoresis if necessary.

VII. INTERPRETATION:

Reported by Lab resident or attending. Utilizing comparison of various patterns to known mobility characteristics and standards assist them in determining the hemoglobin type.

VIII. REFERENCES:

1. Package Insert, "Hemoglobin Electrophoresis Procedure" using Cellulose Acetate Plate in Alkaline Buffer. Helena Laboratories, Beaumont, Texas.

2) U.S. Department of Health and Human Services, Laboratory Methods for Detecting Hemoglobinopathies. Center for Infections Diseases, Atlanta, Georgia. Sept. 1984.

IX. HISTORY:

H-1 This procedure was written by P. Morris on 10-03-97.
H-2 This procedure was revised by P. Morris on 10-08.
H-3 This procedure was revised by S. Richardson on 4/30/12.