Hemoglobin Electrophoresis

What Is a Hemoglobin Electrophoresis Test?

A hemoglobin electrophoresis test is a blood test used to measure and identify the different types of hemoglobin in your bloodstream. Hemoglobin is the protein inside red blood cells responsible for transporting oxygen throughout your circulatory system to your tissues and organs.

If your hemoglobin is healthy, it will transport and release oxygen with maximum efficiency. If it's abnormal in some way, it may cause too little oxygen to reach your tissues and organs.

The types of hemoglobin include the following:

- **Hemoglobin F**: This type is found in growing fetuses and newborns. Soon after birth, it's replaced with hemoglobin A.
- **Hemoglobin A**: This is the most common type of hemoglobin found in healthy children and adults.
- **Hemoglobin C, D, E, M, and S**: These (and many other, rarer variations) are types of abnormal hemoglobin.

The Purpose of Testing

You acquire different abnormal types of hemoglobin by inheriting the genes that produce them. Your doctor may recommend a hemoglobin electrophoresis test to determine if you have a disease that causes the production of abnormal hemoglobin, such as the following.

Sickle Cell Anemia

This disorder is caused by hemoglobin S. Red blood cells become hard and crescent-shaped. They block small blood vessels and prevent blood from circulating properly.

Thalassemias

These genetic disorders can cause the production of too much abnormal hemoglobin and too little hemoglobin A.

Testing Children

Your doctor may also want to test your child if you have a family history of abnormal hemoglobin or they have anemia that's not caused by an iron deficiency.

In the laboratory, a process called electrophoresis passes an electrical current through the hemoglobin in your blood sample. This causes the different types of hemoglobin to separate into different bands. Your blood sample is then compared to a healthy sample to determine which types of hemoglobin are present.

Understanding the Results

Normal Results

The following are healthy levels of hemoglobin in infants and children.

- **Hemoglobin F** (newborn): 50 to 80 percent
- **Hemoglobin F** (6 months): 8 percent
- **Hemoglobin F** (6 months+): 0.8 to 2 percent

The following are healthy levels of hemoglobin in adults.

- **Hemoglobin A**: 95 to 98 percent
- **Hemoglobin A2**: 2 to 3 percent
- **Hemoglobin F**: 0.8 to 2 percent
- **Hemoglobin S**: 0 percent
- **hemoglobin C**: 0 percent

Abnormal Results

If your results show abnormal hemoglobin levels, they may be caused by:

- hemoglobin C disease (a genetic disorder that leads to severe anemia)
- rare hemoglobinopathy (a group of genetic disorders causing the abnormal production or structure of red blood cells)
- sickle cell anemia
- thalassemia

Hb electrophoresis by cellulose acetate paper.

The following buffers and solutions were used for Hb electrophoresis by cellulose acetate paper.

1. Barbitone buffer pH 8.6

Sodium diethyl barbiturate	5.15 g
Barbitone (diethylbarbituric acid)	0.92 g
Distilled water	1000 ml

2. Staining solution

Ponceau S	5 g
Trichloroacetic acid	7.5 g
Distilled water	1000 ml

3. Destainig solution

Acetic acid 5 % (v/v)	50 ml
Distilled water	1000 ml

4. Clearing solution

Glacial acetic acid	125 ml
Methanol	375 ml
Polyethylene glycol	20 ml

5. Absolute methanol

Preparation of Hb electrophoresis by cellulose - acetate paper.

1) Blood prepared according was diluted to 1:4 or 1:5.
2) With the power supply is still disconnected, the compartments of electrophoresis tank were filled with barbitone buffer. The wicks were soaked and positioned in place.
3) The cellulose paper was soaked in a separated dish in barbitone buffer for at least 5 minutes. The paper was immersed slowly to avoid trapping air bubbles and ensure even saturation of the membrane.
4) The membrane was placed between two pieces of absorbent papers.
5) A small volume (10 µl) of each diluted sample was placed into the sample well.
6) The applicator was dipped into the sample wells, and the samples were applied to cellulose – acetate membrane approximately 3 cm from one end of the membrane. The tips of the applicator were allowed to remain in contact with the membrane for 3 seconds.
7) The cellulose – acetate membrane was placed across the bridge of the tank so that the membrane surface is in contact with the buffer, with the line of the cathode end.
8) The power supply was connected and run at 250 – 300 volt for 20 minutes or until a visible separation is obtained.
9) The power supply was disconnected, and the cellulose – acetate membrane was removed and stained in ponceau S for 3 – 5 minutes.
10) The membrane is then removed, drained, and the excess stain was eluted with three changes of destaining solution for 2 minutes each.
11) The dehydration was performed in absolute methanol for 2 – 3 minutes.
12) The membrane then was immersed in clearing solution for 6 – 8 minutes.
13) After clearing the membrane it was dried at 65°C for 4 – 6 minutes.