**INTENDED USE**

Fetal Hemoglobin reagents are used for the acid elution, semi-quantitative determination of fetal hemoglobin in blood smears. Fetal Hemoglobin reagents are for "In Vitro Diagnostic Use."  

As early as 1864, Korber recognized that the hemoglobin of the fetus was more resistant to alkali denaturation than that of the adult. Advances in techniques for protein isolation and characterization led to the discovery that there are several distinguishing properties that make it possible to differentiate fetal from adult hemoglobin. Among these is the resistance of fetal hemoglobin (HbF) to acid elution. When blood smears are immersed in acid buffer, for example, adult hemoglobin is eluted from the erythrocytes, whereas fetal hemoglobin is not. If blood smears are treated in this manner and subsequently stained, erythrocytes having hemoglobin F will take up the stain, while those containing only adult hemoglobin appear as "ghosts". The slide technique for demonstrating fetal hemoglobin in terms of its resistance to acid elution was originally proposed by Kleihauer et al., and later modified by Shepard et al. The Sigma procedure represents a further improvement in this approach as described by Oski and Naiman.  

Fetal hemoglobin estimations are sometimes made to determine possible hemorrhage in the newborn, particularly in cases where there are signs of nectal bleeding. Hemoglobin F assay is also applied to adults as an aid in diagnosing certain types of anemia. For example, from 10–90% fetal hemoglobin is encountered in patients with thalassemia major. Moreover, small increases of fetal blood pigment are usually observed in patients with sickle cell disease.  

It is becoming increasingly common in cases of Rh incompatibility to suppress the proportion of erythrocytes containing fetal hemoglobin in red cell populations. Bull Johns Hopkins Hosp 110:293, 1962  

Sickling occurs in areas of the body where arterial oxygen tension is low. Sickling can cause painful crisis, tissue necrosis, and organ damage. Hemoglobin S disturbs the function of the erythrocyte and the blood, thereby creating sickle cell disease. Thalassemia minor (5–10% fetal hemoglobin) and beta-thalassemia major (40–90% fetal hemoglobin) are the most common causes of sickle cell anemia.  

The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.  

**PROCEDURE**

**STORAGE AND STABILITY:**  
Store Citrate Phosphate Buffer Concentrate in refrigerator (2–6°C). Discard if there is evidence of microbial growth.  

Store Citrate Phosphate Buffer Solution in refrigerator (2–6°C). Stable for 2 weeks. Use a fresh aliquot each day. Discard if there is evidence of microbial growth.  

Store Acid Hematoxylin Solution and Eosin B Solution at room temperature (18–26°C). Solutions may be reused if they are stored in tightly sealed staining jars in subdued light.  

Ethanol fixative should be stored at room temperature. Store tightly sealed and as a flammable liquid. Solution may be reused, but should be discarded if fixation is not adequate.  

Discard Acid Hematoxylin Solution when the time required for suitable staining exceeds 8 minutes.  

Citrate Phosphate Buffer Solution is prepared by diluting 1 volume of Citrate Phosphate Buffer Concentrate with 9 volumes of water.  

Acid Hematoxylin Solution, Eosin B Solution and Ethanol Fixative are ready to use.  

Normal Ranges°  

<table>
<thead>
<tr>
<th>Age</th>
<th>Fetal Hemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 years</td>
<td>0–4</td>
</tr>
<tr>
<td>≥ 2 years</td>
<td>0–2</td>
</tr>
</tbody>
</table>

Excessive values are observed in:  

- Aplastic anemia°  
- Erythremic myelosis°  
- Hemoglobin H disease°  
- Hereditary persistence of hemoglobin F°  
- Hereditary spherocytic anemia°  
- Thalassemia major (40–90% fetal hemoglobin)°  
- Thalassemia minor (5–10% fetal hemoglobin)°  
- Sickle cell anemia°  

The proportion of erythrocytes containing fetal hemoglobin may be estimated several ways. When studying maternal blood for evidence of HbF-containing cells, Oski and Naiman recommended the following:  

1. Count total number of erythrocytes in 5 fields and determine the average number per field.  
2. Count the number of deeply stained HbF-containing erythrocytes in about 30 fields and determine the average number per field.  
3. Calculate percentage of HbF-containing erythrocytes on the basis of the total number of erythrocytes per field.  

Results are reported as the percent HbF present.  

**Sensitivity studies:** According to Oski and Naiman this method is capable of detecting as little as 0.1 mL of fetal blood in maternal circulation.  

**Reproducibility studies:** Using a series of fresh blood specimens, replicate slides were prepared from each and treated with several different lots of stain on separate occasions. Microscopic examination revealed essentially identical results with each blood sample.  

**Correlation studies:** Mixtures of cord blood and compatible adult blood were prepared to yield specimens with HbF concentrations ranging from 26–66%. The blood mixtures were examined by the described technique and assayed chemically by an alkaline denaturation method. The percent HbF values showed an average difference of about 7% between methods. If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.  

**REFERENCES**  
6. Data obtained by Sigma-Aldrich

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Procedure No. 285
Previous Revision: 2005-01
Revised: 2014-09