INTENDED USE

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

As early as 1864, Korber1 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 (hemoglobin F) 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.,1 and later modified by Shepard et al.2 The Sigma procedure represents a further improvement in this approach as described by Oski and Naiman.3

Fetal hemoglobin estimations are sometimes made to determine possible hemorrhage in the newborn, particularly in cases where there are signs of rectal 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 amount of specific gamma globulin, containing anti Rh(D) to be administered, is calculated by assessing the magnitude of fetal-maternal hemorrhage.5 According to described technique, blood smears, which have been properly dried and fixed, are immersed in a citrate buffer pH 3.3 at 37˚C. Adult hemoglobin A (HbA) dissolves out of the cells, whereas fetal hemoglobin (HbF) which is acid resistant, remains intracellular and can be stained for microscopic examination.

REAGENTS

CITRATE PHOSPHATE BUFFER CONCENTRATE, Catalog No. 2851
Sodium citrate, 0.7 mol/L and sodium phosphate, 0.6 mol/L.
ACID HEMATOXYLIN SOLUTION, Catalog No. 2852
Hematoxylin, certified, 1 g/L, aluminum ammonium sulfate, sodium iodate and stabilizers, pH 3.3.
EOSIN B SOLUTION, Catalog No. 2853
Eosin B, 0.1%, aqueous solution. Sodium azide, 0.1%, added as preservative.

STORAGE AND STABILITY:

Store Citrate Phosphate Buffer Concentrate in refrigerator (2–8˚C). Discard if there is evidence of microbial growth.
Store Citrate Phosphate Buffer Solution in refrigerator (2–8˚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.

DETERIORATION:

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

PREPARATION:

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.

PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Either capillary or venous blood may be used. Capillary blood may be transferred directly to a clean microscope slide. Venous blood should be added to a tube containing EDTA or oxalate. For convenience, use 1–2 drops of 2% EDTA Solution, Catalog No. 2854, per mL of blood (1 drop = 1 mg). Although blood-EDTA mixtures have been reported to be satisfactory for 1–2 weeks,1 other studies have concluded that such mixtures should be tested promptly.4 Smears should be prepared within 24 hours from blood collected in oxalate. Using samples from the newborn, it is recommended that the blood be diluted with 0.85% saline, since such specimens have a high content of HbF. Blood smears are not stable and must be tested immediately after preparation.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Microscope
Microscope slides, cover slips
Staining rack/Coplin jars
Water bath, 37˚C
Ethanol Fixative, Catalog No. 2858, 80% v/v ethyl alcohol

NOTES:

For quality control purposes, it is recommended that blood from a normal adult (HbA) and from a newborn or infant (HbF) be included in each series of tests. Barr and Shaffer2 report that fixed positive control slides from cord blood in EDTA can be preserved up to 1 year at –20˚C, in a sealed cardboard box. However, EDTA negative controls do not elute completely after being stored for more than 2 months at –20˚C. These investigators suggest preparing positive and negative smears on the same slide; thus, providing clear and rapid contrasts as reference in reading test slides.

Normal Ranges

<table>
<thead>
<tr>
<th>Age</th>
<th>Percent HbF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 years</td>
<td>0–2</td>
</tr>
<tr>
<td>&gt; 2 years</td>
<td>&lt; 0.1%</td>
</tr>
</tbody>
</table>

Excessive values are observed in:

Aplastic anemia6
Erythremic myelosis7
Hemoglobin H disease8
Hereditary persistence of hemoglobin F9,10
Hereditary spherocytic anemia8
Thalassemia major (40–90% fetal hemoglobin)7
Thalassemia minor (5–10% fetal hemoglobin)7
Sickle cell anemia6

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:

1. Citrate Phosphate Buffer Solution should be warmed to 37˚C in a Coplin jar or staining dish.
2. Using clean, labeled microscope slides, make thin blood smears. Prepare CONTROL slides using positive HbF blood (cord-blood) and normal adult blood. Air dry approximately 10 minutes.
3. Fix slides by immersing in Ethanol Fixative for 5 minutes, rinse thoroughly with tap water and air dry.
4. Immerse TEST and CONTROL slides in pre-warmed Citrate Phosphate Buffer Solution at 37˚C for 5 minutes. Agitate after 1 and 3 minutes of immersion. Degree of agitation may be varied to achieve most desirable results. Rinse thoroughly with distilled water and air dry completely to avoid staining artifacts.
5. Stain the slides for 3 minutes in Acid Hematoxylin Solution. Rinse slides with distilled water and shake off excess water.
6. Counterstain slides for 4 minutes in 0.1% Eosin B Solution. Rinse thoroughly with distilled water and air dry.
7. Place dry coverslip on slide and examine using oil immersion (1000X). The absence of HbF is evident by the presence of ghost cells while retained HbF causes cells to appear bright red. Do not apply oil directly to slide.

NOTE: The 400X magnification may be used, but the resulting larger field may be more difficult to count.

PERFORMANCE CHARACTERISTICS

The proportion of erythrocytes containing fetal hemoglobin may be estimated several ways. When studying maternal blood for evidence of HbF-containing cells, Oski and Naiman5 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 Naiman5 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.5 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%.3 The blood mixtures were examined by the described technique and assayed chemically by an alkali denaturation method.6 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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