

## BIBLIOGRAPHY

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3. Clayton, E.M., Feldhaus, W.D., Phythyon, J.M., Am. J. Clin. Path. 40; 487, 1963.
4. Shepard, M.K., Weatherall, D.J., Conley, C.L., Bull. Johns Hopkins Hosp. 110: 293, 1962.
5. Technical Manual. American Association of Blood Banks, 16th ed. Arlington (VA) 2008, pp 936-937.
6. Henry, R.J., Clinical Chemistry, Principles and Technics, 2nd ed. Harper and Row, Hagerstown (MD), 1974, p 1180.
7. Miale, J.B., Laboratory Medicine Hematology, 6th ed. C.V. Mosby, St. Louis (MO) 1982, p 608.
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## ORDERING INFORMATION

Kit No.	CONTENTS-PRODUCT NO.		
	Fixing SOLN. 101-10	Buffer 101-20	Stain SOLN. 101-30
ST 101-A	1 X 120 ml	1 X 120 ml	1 X 120 ml
ST 101-B	2 X 120 ml	2 X 120 ml	2 X 120 ml
ST 101-C	3 X 120 ml	3 X 120 ml	3 X 120 ml

## OPTIONAL

PROD. NO.	DESCRIPTION
KB-RM	KLEIHAEUER-BETKE FETAL HEMOGLOBIN REFERENCE MANUAL
ST 201	FETAL HEMOGLOBIN CONTROLS

CALL 1-800-678-8933  
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Rev. 11/2011



PROCEDURE NO. 101

## FETAL HEMOGLOBIN

### FOR THE IDENTIFICATION OF FETAL ERYTHROCYTES IN THE PRESENCE OF ADULT RED BLOOD CELLS

#### HISTORY AND PRINCIPLE OF TEST

The detection of fetal erythrocytes in the maternal circulation was first described in 1957 by Kleihauer and associates. (1) The passage of erythrocytes from an Rh positive fetus into the circulation of an Rh negative mother results in the formation of specific Rh antibodies. In subsequent pregnancies, the Rh antibodies formed in the blood serum of the Rh negative mother are readily transmissible through the placenta into the circulation of the fetus. The action of the antibodies on the Rh positive cells of the fetus may result in a disease entity recognized as iso-hemolytic disease, or erythroblastosis. (2)

Rh negative mothers who have been sensitized to the Rh factor as a consequence of transfusion with Rh positive blood from the fetus are administered specific gamma globulin containing anti Rh<sub>0</sub>(D) to suppress the immune reactions. The amount of gamma globulin administered is calculated by assessing the magnitude of fetal/maternal hemorrhage.

The procedure described herein is a modification of that described by Clayton, et al (3) whereas blood smears, which have been properly dried and fixed, are immersed in a citrate/phosphate buffer, pH3.2. Adult hemoglobin (HbA) dissolves out of the cells, whereas fetal hemoglobin (HbF) which is acid resistant, remains intracellular and is stained and enumerated by microscopic examination.

## REAGENTS

For in Vitro Diagnostic Use

### RED CELL FIXING SOLUTION, PRODUCT NO. 101-10

Ethanol, 80%(v/v)denatured

### CITRATE/PHOSPHATE BUFFER, PRODUCT NO. 101-20

0.2 mol/L

Preservatives

### HEMOGLOBIN STAINING SOLUTION, PRODUCT NO. 101-30

Erythrosin, 0.1%

Stabilizers

**PREPARATION:** Reagents are provided ready for use.

### **INSTRUMENT AND MATERIALS REQUIRED**

Microscope	Coplin jars
Microscope slides	0.85% Saline
Small glass test tubes. (12 x 75 mm)	

### **REAGENT STORAGE AND STABILITY**

Reagents are stored between 20-25°C and stable for the period indicated on the label. Reagents may be reused until deterioration in the quality of the slides are noted. Do not pour used reagent back into original containers.

## EXPECTED VALUES (4, 6, 7)

Virtually all adult red blood cells will appear as ghost cells. Virtually all fetal red blood cells will stain a dark reddish-pink. Platelets will stain pink but are usually smaller with spike like projections. Lymphocytes may stain pink but can be distinguished from fetal RBCs by their granular appearances.

The concentration of fetal hemoglobin, as a percentage of total, ranges from 64-95% at birth to approximately 5% at 6 months of age. The percentage of fetal hemoglobin in adults is normally <1%.

Hemoglobin F may occur in relatively high concentrations in adult patients with certain inherited disorders of erythropoiesis such as thalassemia major and sickle cell anemia. In adults with "hereditary persistence of fetal hemoglobin", the concentration of HbF is approximately 26 % of total. Red blood cells in adults possessing these and other hemoglobin abnormalities will stain with varying intensities depending upon the concentration and distribution of the hemoglobin F.

## PERFORMANCE CHARACTERISTICS

### REPRODUCIBILITY:

Ten replicate assays performed by the procedure described herein on two fresh blood specimens containing mean % fetal cells of 0.839 and 30.318 respectively yielded coefficients of variation of less than 1%.

### CORRELATION:

Fifty-three specimens containing either adult whole blood or mixtures of cord blood and compatible adult blood were assayed for fetal cells using the procedure described herein and by a similar commercially available method. The resulting correlation coefficient was 0.9989 and the regression equation had a slope of .09907 and a y-intercept of 0.0138% fetal cells. Mean % fetal cells obtained by the described procedure and the reference method were 8.8 and 8.9 respectively.

### SENSITIVITY:

According to Oski and Naiman (8) the Kleihauer-Betke staining procedure is capable of detecting as little as 0.1 ml of fetal blood in maternal circulation.

## CALCULATION AND RESULTS

Results are expressed as % Fetal Cells

The American Association of Blood Banks recommends the following calculation be used. (5)

1. Count the total number of adult and fetal erythrocytes in as many fields as required to give a total count of at least 2000 cells.
2. Calculate percent fetal cells in the total counted.

### Example:

Total RBCs counted = 2160

Total Fetal RBCs counted = 10

% Fetal Cells =  $10/2160 \times 100 = 0.46$

## RhIG Dosage

The number of Vials of RhIG necessary to protect against Rh immunization is based on the volume of fetomaternal hemorrhage and is calculated as follows.

1. Volume of fetomaternal hemorrhage (FMH) = %fetal cells x 50\*
2. Doses of RhIG required = FMH / 30\*\*

\*5000ml (mothers arbitrarily assigned blood volume)

\*\* one 300 ug dose of RhIG will protect against a transplacental hemorrhage of 30ml of fetal blood

### Example

If % fetal cells = 0.80; FMH -  $0.80 \times 50 = 40$  ml;

Doses of RhIG require =  $40 / 30 = 1.3$  doses (give 2 doses)

Note: When the number to the right of the decimal point is less than 5, round down and add on dose of RhIG. When the number to the right of decimal point is 5 or greater, round up to the next number and add one dose of RhIG, i.e. 2.9 doses (calculated) requires 4 doses.

## SPECIMEN COLLECTION AND STORAGE (3,4)

**BLOOD:** Maternal blood collected with the aid of EDTA or oxalate should be used. Samples should be stored at 2-8°C until assayed. Blood-EDTA mixtures have been reported to be satisfactory for use up to 2 weeks when stored refrigerated. However, it is recommended that such mixtures should be assayed as promptly as possible. Smears should be prepared within 24 hours form blood collected in oxalate. Hemolyzed samples are unsuitable for use.

**AMNIOTIC AND OTHER BODY FLUIDS:** The presence of fetal erythrocytes in amniotic and other body fluids may be assessed by substituting the fluid for blood in the procedure. It will be necessary to isolate the red cells by washing with saline and adding protein (albumin) to the final suspension.

**CORD BLOOD:** Cord blood is fetal blood and is not suitable for assessing fetal/maternal hemorrhage. Cord blood is used for the preparation of positive control material.

## QUALITY CONTROL

For quality control purposes, it is recommended positive and negative control slides be included in each series of tests. CLIA recommends a high and a low-positive control be run. High and low positive and negative controls may be obtained from Sure-Tech Diagnostics as Product No ST 201 (see ordering information). High and low positive controls may also be prepared in the laboratory by adding cord blood to adult whole blood. These spiked controls are then diluted with saline and assayed in the same manner as the patient sample. The appearance of the negative control should be recorded but need not be counted. Note: Fixed slides are stable for up to 30 days stored in the freezer. Control slides may be prepared in advance. When they are needed, remove from freezer, allow to come to room temperature and start the procedure with Step 7. DO NOT RE-FIX

## QUALITY ASSURANCE

Under the CLIA '88 GUIDELINES, laboratories performing the Kleihauer-Betke Fetal Hemoglobin Assay must verify the accuracy of their results at least twice yearly.

## TEMPERATURE:

The Red Cell Fixing Solution, Citrate/Phosphate Buffer and Hemoglobin Staining Solutions must be at an appropriate temperature before using.

Package insert for procedure No. 101 (Rev. 03/2011) stipulates that the procedure is to be run at room temperature. Historically room temperature for clinical labs has been approximately 25°C, i.e. 25°C ± 2°C. However in recent years with the increased use of automated instrumentation, the temperature in the labs may be significantly less than what was considered room temperature. Specifying that a procedure be run at room temperature and assuming the temperature will be approximately 25°C is no longer valid. This is the case with Sure-Tech Procedure No. 101. The temperature maintained in some labs is well below the temperature for the procedure to perform satisfactorily.

Actually, Sure-Tech Fetal Hemoglobin Procedure No. 101 may be run at any temperature providing sufficient quantity of adult hemoglobin is eluted from the adult cells in the specified period of time. This will cause the adult cells to stain a light pink in contrast to the fetal cells which will stain a dark reddish-pink.

As a guide, if the temperature in your lab is between 20°C and 23°C, the temperature of the Citrate Phosphate Buffer (No. 101-20) may need to be adjusted to 23°-27°C. If the temperature in your lab is below 20°C, the temperature of all reagents must be adjusted to 23°-27°C. DO NOT CHANGE THE TIMES. There are many ways to adjust the temperature such as a warm water bath, light bulb, etc. Each lab must decide which method best fits their needs.

## PROCEDURE:

1. Mix the blood sample by gentle inversion.
2. Place 3 drops of 0.85% saline and 2 drops of blood into a glass test tube, mix gently.
3. Place one drop of diluted blood on a glass slide near one end. Prepare a smear by drawing the edge of another slide through the drop and across the slide.

4. Air dry the slide at room temperature.
5. Place the slide in a Coplin jar containing sufficient Red Cell Fixing Solution to cover the smear. Raise and lower the slide 2 or 3 times for even distribution of the fixing solution and allow the slide to remain in the solution at an appropriate temperature for 5 minutes.
6. Remove the slide from the fixing solution, rinse thoroughly with deionized water and air dry.
7. Place the dry slide in a Coplin jar containing sufficient Citrate/Phosphate Buffer to cover the smear. Raise and lower the slide 2 or 3 times for even distribution of the buffer and allow the slide to remain in the solution at an appropriate temperature for 10 minutes.
8. Remove the slide from the buffer solution and blot excess buffer from the slide.
9. Place the wet slide in a Coplin jar containing sufficient Hemoglobin Staining Solution to cover the smear. Raise and lower the slide 2 or 3 times for even distribution of the stain and allow the slide to remain in the stain at an appropriate temperature for 3 minutes.
10. Remove the slide from the Hemoglobin Stain Solution, rinse thoroughly with deionized water and allow to dry at room temperature.
11. Slides must be examined by using oil immersion. Fetal cells will stain a dark reddish-pink while adult cells will appear white to light pink with a slightly darker center. Other cells may also stain to a varying degree and these cells must be identified so as not to be counted as fetal cells. Refer to Sure-Tech Diagnostics Kleihauer-Betke Fetal Hemoglobin Reference Manual for assistance in fetal cell identification.

### Note:

1. In addition to using Coplin jars, most staining racks are acceptable as well as flooding the slides.