

Fetal-Maternal Hemorrhage Determination...Heading 1

Identification of Fetal Hemoglobin (Kleihauer-Betke Test)...Normal

I. Principle: ...Sec. head

The fetal hemoglobin test is based upon the ability of fetal hemoglobin to resist acid elution in contrast to adult hemoglobin, which elutes when exposed to acid buffer. After elution, erythrocytes having hemoglobin F will take up the eosin stain, while those containing only adult hemoglobin appear as "empty ghosts". ...Sec. text

II. Clinical Significance: ...Sec. head

The differential staining is used to estimate the volume of fetal maternal hemorrhage and, in appropriate cases, determine the dose of Rh immune globulin. ...Sec. text

III. Specimen: ...Sec. head

Maternal blood (not cord blood) must be used. Blood must be drawn as soon after delivery as possible to minimize the effect of decreasing fetal RBCs especially when ABO incompatibility exists. A minimum sample of one ml of EDTA anticoagulated whole blood is required. Perform analysis promptly. Refrigerate the specimen until analysis can be performed. ...Sec. text

Note: Notify a Pathologist when the test is ordered. ...Sec. text (soft return, bold, dark yellow)

IV. Materials: ...Sec. head

1. Miniprep ...Sec. text. 1
2. Microscope ...Sec. text. 1

Note: Our defacto standard for.......Sec. text. 1 (soft return, bold, dark yellow)

V. Reagents: ...Sec. head

1. Fetal Hemoglobin kit (Sigma #285) ...Sec. text. 1
 - A. Citrate Phosphate Buffer Concentrate: ...Sec. text. 1A
Stable when stored in a refrigerator (2 - 6°C). Discard if there is any visible evidence of microbial growth. ...Sec. text. 1A

Note: Do not shake the bottle. Chloroform globules at the bottom should not be disturbed. ...Sec. text. 1A (soft return, bold, dark yellow)

■ *Warning: Highly toxic* ...Sec. text. 1A (soft return, insert picture bullet, italic)

VI. Standardization: N.A. ...Sec. head

VII. Procedure: ...Sec. head

1. Preparation and processing of the blood smears: ...Sec. text. 1
 - A. Using the miniprep in the hematology lab, prepare at ...Sec. text. 1A
Note: Wipe blade of miniprep ...Sec. text. 1A (soft return, bold, dark yellow)
 - a. Specimen for positive control ...Sec. text. 1Aa
 - b. For negative control smears ...Sec. text. 1Aa
 - B. Air dry the smears. ...Sec. text. 1A
2. Elution and staining: ...Sec. text. 1
Note: Leave some smears of patient ...Sec. text. 1 (soft return, bold, dark yellow)
 - A. Fix smears by immersing in 80% Ethanol for 5 minutes. ...Sec. text. 1A
 - B. Thoroughly rinse the smears in fresh tap water contained in a Coplin jar. ...Sec. text. 1A
 - C. Completely air dry the smears. ...Sec. text. 1A

VIII. Limitations: N.A. ...Sec. head

IX. Results Derivation: ...Sec. head

1. Reading smears and calculating % fetal RBCs (performed by a Pathologist): ...Sec. text. 1
Note: Record all results on the test ...Sec. text. 1 (soft return, bold, dark yellow)
2. Calc...Sec. text. 1
3. Determination of RhIG dose (by Pathologist-Obste...Sec. text. 1
 - A. Since one 300 µg dose of ...Sec. text. 1A
Example: ...Sec. text. 1A (soft return)
 - a. Kleihauer-Betke reported as 13%...Sec. text. 1Aa
 - b. $13 \times 50 = 65$ ml of fetal blood%...Sec. text. 1Aa
 - c. $65 \text{ ml} \div 30 = 2.2$ doses of RhIG required%...Sec. text. 1Aa

Table: RhIG Dosage for Fetomaternal Hemorrhage ...Table caption

% Fetal Cells	Fetomaternal hemorrhage volume (ml whole blood)		Vials of RhIG to inject
	Average	Ranges	
0.3 - 0.5	20	< 50	2
0.6 - 0.8	35	15 - 80	3
0.9 - 1.0	50	22 - 110	4
1.2 - 1.4	65	30 - 140	5
1.5 - 2.0	88	37 - 200	6
2.1 - 2.5	115	52 - 250	6

...Normal Table

X. Expected Result(s) and/or Critical Values: ...Sec. head

1. Reference: %...Sec. text. 1
less than 0.6% fetal red blood cell's%...Sec. text. 1 (soft return)
less than 30 ml fetal-maternal hemorrhage%...Sec. text. 1 (soft return)
2. Critical: %...Sec. text. 1
greater than 0.6% fetal red blood cells...Sec. text. 1 (soft return)
greater than 30 ml fetal-maternal hemorrhage...Sec. text. 1 (soft return)

XI. Quality Control: ...Sec. head

Note: Record the acceptability of the control slides on the test requisition.

1. Positive Control: ...Sec. text. 1
used to assure the quality of the staining procedure. ...Sec. text. 1 (soft return)
2. Negative Control: ...Sec. text. 1
used to assure complete elution of adult hemoglobin. ...Sec. text. 1 (soft return)

XII. References: ...Sec. head

1. Sigma Package insert, Sigma 285, Revised May 1995. ...Reference text
2. AABB Technical Manual, Chapter 18...Reference text
 - i. July 1986 K. Weiland ...Auth. list
 - ii. June 1989 Dr. Cicciarelli (Revised) ...Auth. list
 - iii. May 1995 B. Lucas (Revised: Finley/SJU staff to elute & stain slides.) ...Auth. list

Comprehensive review:...Sec. text bold

Reviewer: _____ ...Signa. text

Pathologist: _____ ...Signa. text

Technical Director: _____ ...**Signa. text**

Interim review: ...**Sec. text bold**
