Fetal-Maternal Hemorrhage Determination
Identification of Fetal Hemoglobin (Kleihauer-Betke Test)

I. Principle:
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".

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

III. Specimen:
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.

Note: Notify a Pathologist when the test is ordered.

IV. Materials:
1. Miniprep
2. Microscope

Note: Our defacto standard for.

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

      Note: Do not shake the bottle. Chloroform globules at the bottom should not be disturbed.
      Warning: Highly toxic

VI. Standardization: N.A.

VII. Procedure:
1. Preparation and processing of the blood smears:
   A. Using the miniprep in the hematology lab, prepare
      Note: Wipe blade of miniprep
      Specimen for positive control
      For negative control smears
   B. Air dry the smears.

2. Elution and staining:
   Note: Leave some smears of patient
   Fix smears by immersing in 80% Ethanol for 5 minutes.
   Thoroughly rinse the smears in fresh tap water contained in a Coplin jar.
   Completely air dry the smears.
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 x 50 = 65 ml of fetal blood%...Sec. text. 1Aa
      c. 65 ml ÷ 30 = 2.2 doses of RhIG required%...Sec. text. 1Aa

Table: RhIG Dosage for Fetomaternal Hemorrhage …Table caption

<table>
<thead>
<tr>
<th>% Fetal Cells</th>
<th>Fetomaternal hemorrhage volume (ml whole blood)</th>
<th>Vials of RhIG to inject</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 - 0.5</td>
<td>Average 20</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>0.6 - 0.8</td>
<td>35</td>
<td>15 - 80</td>
</tr>
<tr>
<td>0.9 - 1.0</td>
<td>50</td>
<td>22 - 110</td>
</tr>
<tr>
<td>1.2 - 1.4</td>
<td>65</td>
<td>30 - 140</td>
</tr>
<tr>
<td>1.5 - 2.0</td>
<td>88</td>
<td>37 - 200</td>
</tr>
<tr>
<td>2.1 - 2.5</td>
<td>115</td>
<td>52 - 250</td>
</tr>
</tbody>
</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